PROSTAGLANDINS AND UTERO-OVARIAN RELATIONSHIPS IN THE SHEEP

BY

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THESIS

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TO

MY MOTHER & MY FATHER

WITH LOVE AND GRATITUDE

Sabah

The work included in this thesis is all my own with the exception of the radioimmunoassays.

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ABSTRACT

Previous work on the control of luteal function in the oestrous cycle of the sheep is assessed and discussed. The experimental work produced the following conclusions:-

1. No difference was found between $PGF_2\alpha$ and PGE_2 content and synthesizing ability of the uterine caruncular and non-caruncular endometrial tissue; but the uterine myometrial tissue contained significantly less and produced less $PGF_2\alpha$ than did the endometrial tissues.

2. A significantly higher $PGF_2\alpha$ content was found in the second half of oestrous cycle than in the first half.

3. No significant changes was found either in the content or synthesizing ability of PGE_2 and 6-oxo-$PGF_1\alpha$ of the different uterine tissue indicating that these compounds are probably not involved in luteolysis.

4. Plasma concentrations of $PGF_2\alpha$ were episodic in nature and showed an increase at about day 12 and 13 of the oestrous cycle. The largest release of $PGF_2\alpha$ occurred on the day after progesterone secretion by the CL had ceased. These findings appear to support the involvement of $PGF_2\alpha$ in luteolysis but also suggest that a major role for $PGF_2\alpha$ at the end of the cycle may be to complete luteolysis and to prevent any functional recovery of the CL.

5. A significant relationship was found between the ability of endometrial tissue to synthesis $PGF_2\alpha$ and PGE_2 and between the endometrial content of $PGF_2\alpha$ and PGE_2. This suggests that either PGE_2 production is a by-product of $PGF_2\alpha$ production or that it is the availability of a common pre cursor that controls the synthesis of the two prostaglandins. However the availability of arachidonic acid was not a limiting factor.

6. It was found that the endometrial synthesizing ability for $PGF_2\alpha$ was significantly increased 3 and 2 days before the onset of oestrus in sheep with an ovary adjacent to the uterine tissue sampled but not in those animals with the adjacent ovary removed. This indicates that the presence of an ovary adjacent to the uterine horn is necessary for the normal manifistation of the $PGF_2\alpha$ synthesizing ability of the endometrium and suggests that the ovary exerts a local influence over endometrial $PGF_2\alpha$ synthesizing ability in the adjacent uterine tissue.

7. No relationships was found between plasma concentrations of $PGF_2\alpha$ and its endometrial content and synthesizing ability, thus implying that the release of $PGF_2\alpha$ is under independent control.

8. A significant relationship was found between the concentrations of $PGF_2\alpha$ and progesterone in the uterine venous blood. This was most demonstrable when $PGF_2\alpha$ levels were compared with the

progesterone level half-hour previously. This suggests that increased $PGF_2\alpha$ occurs in response to an increase in progesterone secretion and $PGF_2\alpha$ may thus play an important role in retaining the progesterone secretion at optimum during the luteal phase of oestrous cycle.

9. Contrary to expectations, the anastomosis of the utero-ovarian vein to the anterior mammary vein but leaving the ovary in situ was found not to interrupt the normal oestrous cycle. This suggests the involvement of another route in the transfer of $PGF_2\alpha$ to the adjacent ovary in addition to the normal route through the counter-current mechanism of transfer from the utero-ovarian vein to the ovarian artery.

10. Significantly higher concentrations of $PGF_2\alpha$ were found in the oviducal vein and the ovarian vein as well as in the uterine vein when compared to peripheral levels. Thus the alternative route is probably via the oviducal vein and then by transfer from the ovarian vein to ovarian artery in the ovarian pedicle.

11. Daily injection of progesterone to sheep was found to lead to accumulation of large amount of fluid rich in $PGF_2\alpha$ in the uterine lumen. Concentrations of $PGF_2\alpha$ higher than peripheral were also found in the uterine venous blood of these animals. These findings show that a high concentration of progesterone can cause $PGF_2\alpha$ release.

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TERMS USED

For brevity the following terms will be used.

Utero-ovarian and Ovarian Vein

The term ovarian vein has been recommended by an International Committee (1968) for the common vein which drains the uterus and the ovary. However in the present study the old term "utero-ovarian vein" will be used for the common vein carrying both the ovarian and uterine venous blood to emphasize that it contains a high proportion of uterine blood. The term "ovarian vein" has been restricted to the ovarian branch of the "utero-ovarian vein", above the bifurcation with the main uterine drainage.

Luteotrophic & Luteolytic

The terms "luteotrophic" and "luteolytic" have been used in their broadest sense to mean capable of maintaining or destroying the corpus luteum and/or its function.

Isolated Uterine Horn

The term "isolated uterine horn" refers to a uterine horn which has been ligated and transected at the junction of the horn and the body of the uterus.

Day 1 of the Oestrous Cycle

The oestrous cycles of animals have been expressed using the notation that the first day of oestrus is day 1.

ABBREVIATIONS

The following abbreviations will be used.

CL	Corpus luteum
FSH	Follicle-stimulating hormone
GH	Growth hormone
GnRH	Gonadotrophin-releasing hormone
HCG	Human chorionic gonadotrophin
IUD or IUDs	Intrauterine device or intrauterine devices
LH	Luteininzing hormone
LH-RH	Luteinizing hormone - releasing hormone
$PGF_{2\alpha}$, PGE_2 etc.	Prostaglandin $F_{2\alpha}$, Prostaglandin E_2 etc.
PMSG	Pregnant mare's serum gonadotrophin

INTRODUCTION

In 1916 Loeb reported the occurrence of degenerative changes in the corpus luteum (CL) of the guinea-pig ovary just before the initiation of a new oestrous cycle. A few years later, he (Loeb, 1923a, b) showed that the uterus played an important role in controlling the life-span of the CL and even suggested that the uterus produced an internal secretion which exerted an abbreviating effect on the CL (Loeb, 1927). However subsequent research came to be focussed on the influence of the ovary on the uterus and the pituitary control of ovarian functions. In the last few years research workers have returned to Loeb's line of enquiry and have shown a luteolytic function of the uterus on the life-span of CL.

The present thesis deals with these utero-ovarian relationships in the non-pregnant sheep, and attempts to throw more light on the factors controlling the uterine influence on luteal function.

The first part of this thesis consists of an interpretation and discussion of the relevant literature on the control of the function of the CL of the sheep. The growth and development of the CL is considered first followed by the various influences that manipulation of the uterus can exert over these endocrine bodies, such influences include foreign bodies and toxic agents in the uterus and removal of the uterus. Next, the pathway whereby these effects occur is considered, as are attempts to extract active substances. Non-uterine factors influencing luteal functions, such as the embryo, the pituitary gland and ovarian steroids are then considered. To complete the picture the identity of the uterine luteolytic hormone

as prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is discussed.

The second part of the thesis consists of experiments performed by the author which extend our understanding of the factors controlling $PGF_2\alpha$ synthesis and release, and hence the control of luteal function by the uterus.

REVIEW OF PREVIOUS LITERATURE

THE CORPUS LUTEUM OF THE OESTROUS CYCLE

After ovulation, a dramatic change occurs in the ovarian follicle which results in the formation of a true endocrine gland within the cortex of the ovary, called the corpus lutuem (CL). These luteal cells contain secretory granules and the yellow pigment from which the name corpus luteum is derived (i.e. yellow body).

The newly formed CL in the sheep increased rapidly in size between the 36th hour and the 5th day after ovulation. This growth continues more slowly between the 5th and 10th days and the CL reaches its maximum size of 10 to 11 mm diameter during this period. About the 12th day the CL begins to regress in size and shows a marked reduction by day 16. This regression accelerates just before the time of the next period of oestrus (Quinlan & Maré, 1931; Grant, 1934; Warbitton, 1934; Deane, Hay, Moor, Rowson & Short, 1966; Hutchinson & Robertson, 1966). The secretory acitivity of the ovine CL is not fully developed until about the 7th day after oestrus (Edgar & Ronaldson, 1958). These authors assayed the progesterone level in the blood of ewes. Due to the low sensitivity of their assay no progesterone was detected in the blood from the jugular vein of any ewe, but progesterone was found in venous blood draining the active ovary. A detectable amount of progesterone appeared on the 3rd day and the mean concentration increased to about 1.8 $\mu g\ ml^{-1}$ on the 7th day. This concentration was maintained until the 15th day and fell to $<0.15\ \mu g\ ml^{-1}$ on the last day of the cycle when luteal degeneration in the non-pregnant ewe was almost complete

(Edgar & Ronaldson, 1958). Numerous later workers have confirmed this trend in progesterone secretion (see p.62). These results demonstrated that the CL in the sheep appeared to reach its maximum size and full secretory activity on about the 7th day after the onset of oestrus. Although the size of the CL may not necessarily be related directly to its secretory activity, the significant decrease in size which occurs some time between the 10th and 15th day after the onset of oestrus, suggests that the progesterone output of the ovary may be dropping by this time (Hutchinson & Robertson, 1966). The high progesterone secretion during the cycle causes inhibition of the later stage of follicular growth, while the decrease of progesterone output at the end of the cycle removes this inhibition and allows follicular growth and ovulation. This is illustrated by the result of McKenzie & Terrill (1937); Dufour, Ginther & Casida (1971a), who found that surgical removal or ablation of the corpora lutea in sheep resulted in the early initiation of a new heat, ovulation or both. Much information on the control of the CL has come from various experimental manipulations of the reproductive tract of the ewe.

THE EFFECTS ON LUTEAL FUNCTION OF FOREIGN BODIES IN THE UTERUS OF THE SHEEP

Reproductive changes induced by foreign bodies inserted into the uterus is currently an important topic for all interested in population control. For several centuries Arab and Turkish camel owners have inserted small round stones into the uterus of their animals in order to prevent pregnancy, but it is only recently that Intra-Uterine Devices (IUDs) have been proved effective for prevention of conception in humans (Bland & Donovan, 1966). The effect of IUDs in sheep has been looked at by a number of workers and sheds some light on luteal control.

Moore & Nalbandov (1953) showed that the insertion of paraffin wax or plastic beads of about 10 mm diameter into the uterus of the sheep during the early luteal phase (day 3) of the oestrous cycle causes a marked decrease in the mean length of the oestrous cycle (from 16.1 to 12.9 days). Insertion of smaller beads (8 mm diameter) caused a more marked shortening of the cycle (i.e. to 11.7: $p<0.05$), and removal of the beads caused resumption of normal 16-day cycle. However, if the bead-containing section of the uterus was "denervated", the presence of the plastic bead had no effect upon cycle length. Nalbandov, Moore & Norton (1955) found that when the bead was inserted during late luteal phase (day 8 i.e. before the time of implantation), the cycles were significantly prolonged (from 16.3 days to a mean of 23.1 days). Again as with insertion on day 3, the modification of the cycle length was prevented if the area of uterus containing the

bead was "denervated". Insertion of the plastic bead into the uterus of the sheep on day 13 neither shortened nor lengthened the cycles. Only the time of operation differed between the three groups. Inskeep & co-workers, (Oloufa, Inskeep, Pope & Casida, 1961; Inskeep, Oloufa, Howland, Pope & Casida, 1962) confirmed the shortening effect of a plastic bead (7 mm) placed in the uterus of sheep on the third day of the oestrous cycle but were unable to substantiate the lengthening effect of insertion on day 8. Inskeep's group found that ovulation without overt oestrus was frequent in the treated animals. Thus as Nalbandov et al. (1955) did no relaparotomize the day 8 animals until day 23 and neither did they mark the original CL, it is possible that the occurrence of silent heats could explain the extended cycles. Thus a combination of a normal length cycle with a shortened cycle but not separated from it by an oestrous period would explain the 23-day cycle. Other than the delay in return to oestrus, no evidence was obtained that implantation of a bead into the uterus on day 8 caused persistence of CL.

The results of Ginther, Pope & Casida (1965, 1966) showed that insertion of a polyethylene plastic coil (15 mm diameter, 200 mm length) into one uterine horn on day 4 of the oestrous cycle can cause premature regression of the corpora lutea in the ipsilateral ovary but not in the opposite ovary. When corpora lutea were only present in the ovary adjacent to the operated uterine horn, the luteolytic influence of the coil reduced the length of the oestrous cycle. Ovulation from both ovaries or from only the ovary adjacent

to the unoperated horn results in a normal length cycle. Furthermore, Stormshak and co-workers, (Stormshak & Hawk, 1966; Stormshak, Lehmann & Hawk, 1967) showed that modification of luteal function occurred irrespective of whether the plastic spiral was in the anterior or the posterior portion of the uterine horn. Stormshak et al. (1967) also found that an intact oviduct was not necessary for the effect of the spiral on luteal function. Unlike, Ginther, Pope & Casida (1966), Stormshak et al. (1967) found that the oestrous cycle during which spirals were inserted was not consistently shortened when the CL were present only in the ovary adjacent to the operate uterine horn, although subsequent cycles were shortened. However, Strakosch, Hecker & Wodzicka-Tomaszewska (1973) found that IUDs (8 mm) inserted on day 4 of the oestrous cycle resulted in a dramatic reduction in oestrous cycle length due to curtailed luteal function. Devices of similar size inserted on day 9 of the cycle had no effect on the current cycle in four out of five sheep, but resulted in a dramatic reduction in cycle length in all subsequent cycles in these sheep. Smaller devices (2 mm), inserted on day 4, appeared to have only a borderline effect on cycle length and luteal function. Insertion of nylon threads (0.2 mm diameter) into both uterine horns allowed approximately normal cycle lengths and normal peripheral progesterone concentrations. Like Moore & Nalbandov (1953) they concluded that the effect of the IUDs on luteal function is dependent on distension of the uterine horns. Wodzicka-Tomaszewska, Hecker & Bray (1973) studied the effect of the day of insertion of 8 mm spiral plastic coils on luteal function and cycle length and found that insertion of IUDs up to day 4 induced premature luteolysis, whereas

insertion at day 5 or later allows normal luteal development and may even prevent luteolysis at the normal time. The change from a luteolytic to luteotrophic effect at day 5 coincides with the beginning of the secretion of progesterone by the CL.

The denervation experiments of Moore & Nalbandov (1953) led them to postulate a primary role of the pituitary in the luteolytic effect of IUDs. However, their method of denervation (i.e. by completely severing a segment of the cornu, about 30-34 mm in length, from the rest of the uterus and from all supporting structures, rotating it and then suturing the resected part to the cut surfaces in order to assure re-establishment of the circulation), achieved more than just interrupting neural pathways, the vascular and lymphatic pathways were also interrupted. Furthermore, their experiments with stalk-section and cutting the uterine splanchnic nerves (Nalbandov & St. Clair, 1958) do not convincingly indicate the existence of a neural pathway. Certainly, Ginther, Hawk & Casida (1966) found that the insertion of a large IUD resulted in high luteinizing hormone (LH) activity in the anterior pituitary both at oestrus and postoestrus. But as Stormshak and co-workers (Stormshak & Hawk, 1966; Stormshak, Lehmann & Hawk, 1967) found that the administration of 1600 i.u. of human chorionic gonadotrophin (HCG) from day 4 to 6 of the oestrous cycle counteracted the luteolytic effect of the spiral and as LH appears to have only luteotrophic properties in the sheep (see p.44) the relevance of the finding of Ginther, Hawk & Casida (1966) is obscure (see also p. 85). The insertion of IUD on day 5 was found to

reduce the interovulatory interval only when the IUD was ipsilateral to the CL. However, when inserted on day 2 the interval was reduced whether the IUD was ipsilateral or contralateral (Ginther, 1968a). Moreover, Ginther (1970), showed that the luteolytic effect of an IUD when inserted into the contralateral horn on day 2 was exerted through a local direct pathway to the opposite ovary rather than through the general circulation. The pathway to the opposite side was not through the uterine lumen or the uterine wall, for severance and the ligation of the uterine horn caudal to the IUD did not prevent luteolysis. The results suggest that the most likely pathway to the opposite side was through the intercornual area (Ginther, 1974). The intercornual area in sheep contains many prominent veins which anastomose the left and right uterine venous systems (Ginther, 1974). The hypothesis that the pathway to the opposite side was through venous channels was tested (Ginther & Bisgard, 1972). On day 2 of the oestrous cycle uterine tissue ipsilateral to the CL was removed and IUD was inserted into the contralateral uterine horn. In some of the ewes an anastomosis was made between the main uterine vein on the intact, contralateral side and the corresponding vein on the ipsilateral side. On day 11, CL were weighed and the anastomosis was examined for patency. Mean weights of CL were significantly different among the three experimental groups (patent venous anastomosis, 49 mg; occluded venous anastomosis, 712 mg; sham-operated, 715 mg). These results strongly supported the hypothesis that the pathway to the opposite side was through venous channels and demonstrated that the main uterine vein served as the proximal component of a direct pathway between a uterine horn and adjacent ovary in IUD-treated ewes.

THE EFFECTS ON LUTEAL FUNCTION OF TOXIC AGENTS AND INFLAMMATION OF THE UTERUS IN THE SHEEP

In sheep the speed of response of the uterus to infection varies with the stage of the oestrous cycle. For instance, the leucocytic response to Escherichia coli (Migula) Castellani & Chalmers, and the clearance of the bacteria themselves from the uterine lumen occurred significantly more slowly during the luteal phase than at oestrus (Hawk, Turner & Sykes, 1961). Brinsfield, Hawk & Leffel (1963) compared the leucocytic responses in uteri at oestrus and dioestrus to look for evidence of hormonal effects on the rate of leucocytic passage through the endometrium. They found that most of the E. coli were killed significantly earlier in ovariectomized ewes than in oestrous ones ($p<0.01$), and significantly earlier in oestrous ewes than in dioestrous ones ($p<0.01$).

Hawk, Brinsfield & Righter (1963) measured the vascular, permeability of uterine endometrium and serosa by intensity of tissue blueing after intravenous injection of solution containing 2% trypan blue in 0.87% NaCl. They found that the permeability was high in oestrous ewes; intermediate in ewes in the luteal phase and low in ovariectomized cotrols. Furthermore, during experimental acute infection in the uterine lumen due to E. coli, endometrial vascular permeability increased greatly in the ovariectomized ewes but little or not at all during the luteal phase or at oestrus. Thus endogenous ovarian hormones appear to modify

the vascular response (Hawk, Brinsfield & Righter, 1963). Serosal vascular permeability increased markedly in all groups. Thus it is probable that in sheep, the uterus becomes more susceptible to infection when a CL is present in the ovary (Brinsfield et al. 1963; Hawk et al. 1963).

Brinsfield & Hawk (1967, 1968) found that the introduction of bacterial infection of E. coli into the uterus of sheep approximately 36 hour after detection of oestrus, shortened the oestrous cycle length by causing premature regression of the CL. The bacteria were introduced into the uterine horn adjacent to the ovary containing an ovulation point. The uterine horn was then ligated to prevent drainage of the inoculum from the uterine lumen. Six days after treatment, the induced inflammation in the ligated portion of one uterine horn had inhibited the development of the corpora lutea of the adjacent ovary and resulted in their early regression (CL weight: 93.6 mg; $p<0.01$) in all animals, whereas normal luteal weight were found in those animals receiving similar injections into broad ligament (CL weight: 527.8 mg) or only saline into the uterine lumen (CL weight: 512.8 mg).

Brinsfield, Higginbotham & Hawk (1969) found that the CL development was inhibited and CL regression hastened following inflammation induced by injecting a suspension of E. coli on days 2, 4, 6 or 8 into the lumen of a uterine horn adjacent to an ovary containing a CL. Uterine infection on day 10 did not cause luteolysis. Oestrous cycles were not lengthened when

infection was induced on day 10, 14, 16 but cycle lengths were prolonged in some ewes infected on day 12. Previously Coudert & Short (1966) had also found prolongation in the life-span of CL in six out of eleven sheep in which Corynebacterium pyogenes (Glage) Eberson or Vibrio foetus Smith & Taylor had been inoculated into the uterine lumen at day 10 of the cycle. Fully functional corpora lutea, as determined by progesterone concentration in ovarian vein blood and corpora lutea tissue, were present at day 21 in 3 animals. The uterus of most of the infected animals was not noticeably distended. It would thus appear that the presence of uterine infection can cause either an abbreviation or extension of luteal function. It seems probably that the stage of the cycle at which infection is initiated is the operative factor as to which modification occurs. Infection commencing in the uterus when the resistance is high, may therefore only stimulate the uterus, whereas bacterial invasion late in the cycle may completely inactivate the endometrium thus effecting luteal function in similar manner to hysterectomy (see later).

The introduction of toxic substances into the uterus can also alter luteal activity. Woody, Ginther & Pope (1969) using sheep found that a unilateral intrauterine injection on day 4 of solution containing 45% propylene glycol, 15% ethanol, 15% benzyl alcohol and 25% water, causes regression of the CL only if the treated uterine horn is adjacent to the ovary that contains the CL. Oestrous cycle length was also reduced to only 9 days ($p<0.005$).

However, such injection into the horn adjacent to the ovary not containing the CL, allowed normal 16-day cycles. When the benzyl alcohol in the solution was replaced by ethanol the cycle length was not reduced. It therefore would appear that irritation and inflammation due to benzyl alcohol can cause luteolysis.

THE EFFECTS OF HYSTERECTOMY ON LUTEAL FUNCTION

Total Hysterectomy

The effects of hysterectomy have been extensively reviewed by previous workers (Anderson, Bowerman & Melampy, 1963; Bland & Donovan, 1966; Melampy & Anderson, 1968; Anderson, Bland & Melampy, 1969; Caldwell, Rowson, Moor & Hay, 1969). In general, the removal of the whole sheep uterus in the presence of functional corpora lutea caused maintenance of luteal functions for a period equivalent to or longer than pregnancy (Wiltbank & Casida, 1956; Kiracofe & Spies, 1963; 1964; Moor & Rowson, 1964; Rowson & Moor, 1964; Bolt, Menzies & Spies, 1966, Collins, Inskeep, Howland, Pope & Casida, 1966).

Wiltbank & Casida (1956) demonstrated that the complete removal of the sheep uterus 3 to 8 days after heat prolonged the life-span of the CL for an extended period of time (i.e. as long as 100 days). In fact Kiracofe & Spies (1963) found that when ewes were totally hysterectomized on day 4 to 6 of the oestrous cycle, regression of the CL was estimated to occur between 160 and 170 days postoestrus. Rowson & Moor (1964) also showed that total hysterectomy, completely inhibited cyclical activity by causing the original corpora lutea to be maintained in a functional state. Moor & Rowson (1964) further showed that hysterectomy, even if performed on the 15th day after oestrus, can arrest the involutionary changes in the luteal cells, provided the CL is still functional at the time of hysterectomy.

Similarly, Kiracofe & Spies (1964) showed that removal of the uterus and oviducts on days 12-17 of the oestrous cycle in ewes, maintained the CL life-span to slaughter at day 30-31 in all ewes except one which was in oestrus when hysterectomized on day 17. These results thus suggest that the CL life can be extended by hysterectomy as late as the last day of the cycle.

It would appear from these observations that the uterus plays an essential part in regulating luteal function in the sheep and that the life-span of the CL is not finally determined until the last day of the cycle. The retrogressive changes observed in the CL of the non-pregnant sheep during the final stage of the cycle would appear to be dependent on the continuous luteolytic effect of the uterus at that stage.

Partial Hysterectomy & the Local Effect of the Uterus

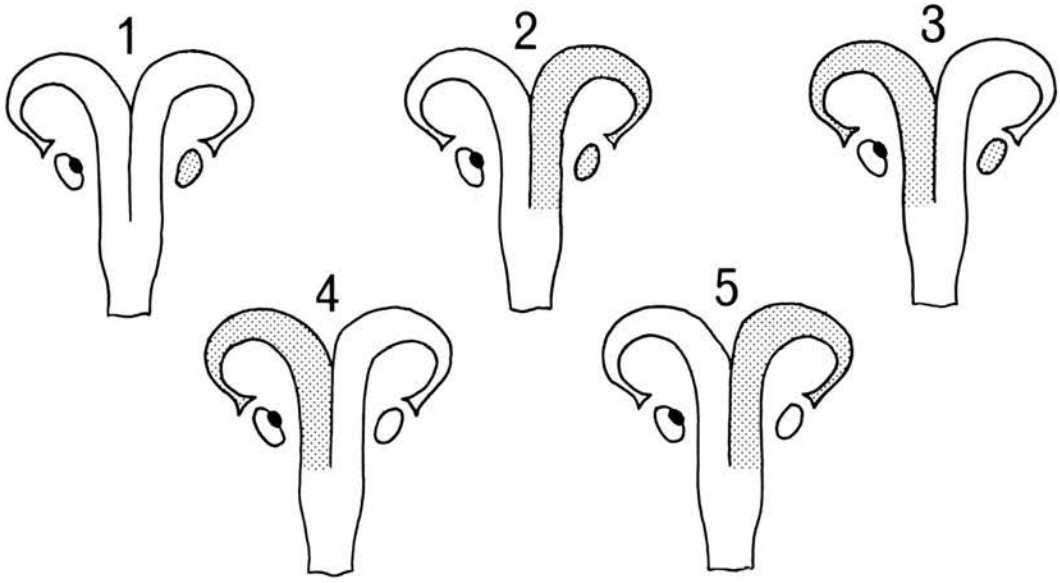
Originally it was thought that following the removal of an increasing proportion of the uterus there was an increase in the life-span of the corpora lutea roughly proportional to the amount of the uterus removed (Rowson & Moor, 1964). However, Wiltbank & Casida (1956) demonstrated that the removal of approximately half of the uterus in the ewe did not prolong the life-span of the CL. While, it was found that cycles, only slightly longer than normal, persisted after removal of all except the cervix and a very small amount of endometrium adjacent to the ovary (Wiltbank & Casida, 1956). It thus appears that a threshold

amount of functional uterus is necessary for the initiation of luteal regression in sheep. The local nature of this phenomenon was elegantly demonstrated by a series of experiments in which the persistence of the CL was shown to be dependent on the quantity of the uterine tissue which was left adjacent to the ovary containing the CL (See Fig 1; Rowson & Moor, 1964 ; Barley, Butcher & Inskeep, 1966 ; Inskeep & Butcher, 1966; Moor & Rowson, 1966a,b). The results of the experiments illustrated in Fig 1 showed that the unilateral ovariectomy alone (Fig 1, group 1) has no effect on the length of subsequent cycle. Unilateral hysterectomy whether alone or in combination with removal of the contralateral ovary consistently prolonged the luteal life-span in the ipsilateral ovary (Fig 1, group 3 & 4) but did not effect the CL in the contralateral ovary (Fig 1, group 2 & 5). Thus the uterus has a local action in producing luteal regression in sheep. A similar local action of hemi-hysterectomy was demonstrated by Caldwell, Rowson, Moor & Hay (1969) following the induction of accessory CL.

A natural example of this phenomenon was described by McCracken & Caldwell (1969) in which no surgical interference had taken place. A ewe, in which the left uterine horn was congenitally absent leaving the left ovary without the normal direct connection to uterine tissue, had not exhibited oestrus for 58 days. At laparotomy it was found that corpora lutea were only present on the side from which the uterine horn was absent.

Figure 1

Diagrammatic representation of the surgical procedures carried out in the five groups of sheep. The stippled area shows the portion of the uterus and the side of the ovary removed in each animal. A large black dot in an ovary indicates the presence of a CL. (After Barley, Butcher & Inskeep, 1966; Inskeep & Butcher, 1966; Moor & Rowson, 1966a,b).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THE EFFECT OF INTERRUPTION OF BLOOD VESSELS BETWEEN THE UTERUS AND THE OVARY ON THE LUTEAL FUNCTION

A lytic factor could find its way from the uterus to the ovary in the fluid secretion of the oviduct (Bellvé & McDonald, 1968). These authors reported that the direction of fluid flow is towards the ovary at the time of luteal regression; however, Moor & Rowson, (1970) found that normal regression of the CL was not prevented by ligation of the oviduct. Local diffusion from the uterus to the ovary through the peritoneal cavity seems unlikely as a normal mechanism due to the local nature of the pathway and the fact that application of endometrial extracts or tissue directly to the ovary of the ewe did not result in regression of the CL (Caldwell et al. 1969).

Another possible route whereby this local influence can get from uterus to the ovary is a blood-vascular one. An early approach to studying the role of blood vessels was to ligate or section veins and arteries between the uterine horn and adjacent ovary. Luteal maintenance after such experimental intervention was taken as an indication that the ligated structure played a role in the local pathway.

In sheep Kiracofe and co-workers (Kiracofe, Spies & Gier, 1963; Kiracofe, Menzies, Gier & Spies, 1966) found that bilateral ligation of the major uterine arteries and veins early in the oestrous cycle resulted in maintenance of corpora lutea in seven of eight ewes. While unilateral ligation resulted in prolonged luteal maintenance in only one of six ewes. The bilateral or unilateral ligation (both

ipsilateral or contralateral to the CL) of the artery alone, excluding the vein, or the unilateral ligation of both artery and vein on the side adjacent to the ovary not bearing the CL, did not effect cycle length. These results suggest that the uterine vein is important in the luteolytic action of the uterus. Not unexpectedly the ligation of major ovarian arteries and veins resulted in regression of corpora lutea in 29 of 37 totally hysterectomized ewes (Kiracofe et al. 1966). Further investigation (Barrett, Blockey, Brown, Cumming, Goding, Mole & Obst, 1971; Restall, Hearnshaw, Glesson & Thorburn, 1973) of the local pathway between the uterus and the ovary in ewes by separation of the ovarian artery from the uterine vein to above the entry of the ovarian veins, showed that the corpora lutea persisted beyond their normal life-span. In ewes in which the separation was incomplete the CL had regressed (Barrett et al. 1971). Furthermore, ligation of the main uterine vein ipsilateral to the ovary which contained the CL not only delayed the regression of the CL, but also inhibited the luteolytic effect of exogenous oestradiol and partially blocked the luteolytic effect of an IUD (French, 1973). In another study (Baird & Land, 1973) the CL was maintained in 4 out of 10 ewes in which the main uterine vein alone was ligated and divided. In those ewes which returned to heat, tubal branches of the uterine vein had formed an anastomosis between the uterine and ovarian veins. Division of these anastomotic veins in addition to the main uterine vein inhibited luteal regression in 6 out of 7 ewes. The presence of these tubal venous arcades of anastomotic vessels may explain the inconsistent results obtained by other workers (e.g. Kiracofe et al.1966), when the main uterine vein and artery were ligated.

The vessel ligation experiments have directed attention to the possible involvement of the blood vessels in the local pathways; however data from such projects must be interpreted with great care. The vascular system is dynamic and readily compensates for intervention, and so may lead to erroneous conclusions. For instance, the relative amount of uterine venous effluent which discharged through the tubal veins must be small in normal ewes, but could be considerable after ligation of the main uterine vein (Ginther, 1974).

Investigations of utero-ovarian vascular and lymphatic connections have failed to indicate specific structures that could function as a portal system between the uterus and the ovaries in sheep (Morris & Sass, 1966). Transport via the lymphatics appears to be unlikely since several studies have shown that the uterine lymphatics do not pass to the ovary (Morris & Sass, 1966; Meckley & Ginther, 1969). Moreover the results of Baird & Land (1973) showed that normal luteal regression occurred in all ewes in which the main uterine vein was left intact but all other connection between the ovarian pedicle of the ovary containing CL and the adjacent uterine horn were divided. These findings confirm that neither the oviduct nor the uterine artery and its branches (Kiracofe et al. 1966) are essential for a luteal regression and suggest that lymphatics and neural connections are likely to be unimportant. However, with regard neural pathways the work of Restall et al. (1973) must be borne in mind. They found that guanethidin sulphate blocked the effect of luteolysin in 3 of 4 sheep, thus suggesting the involvement of a sympathetic pathway.

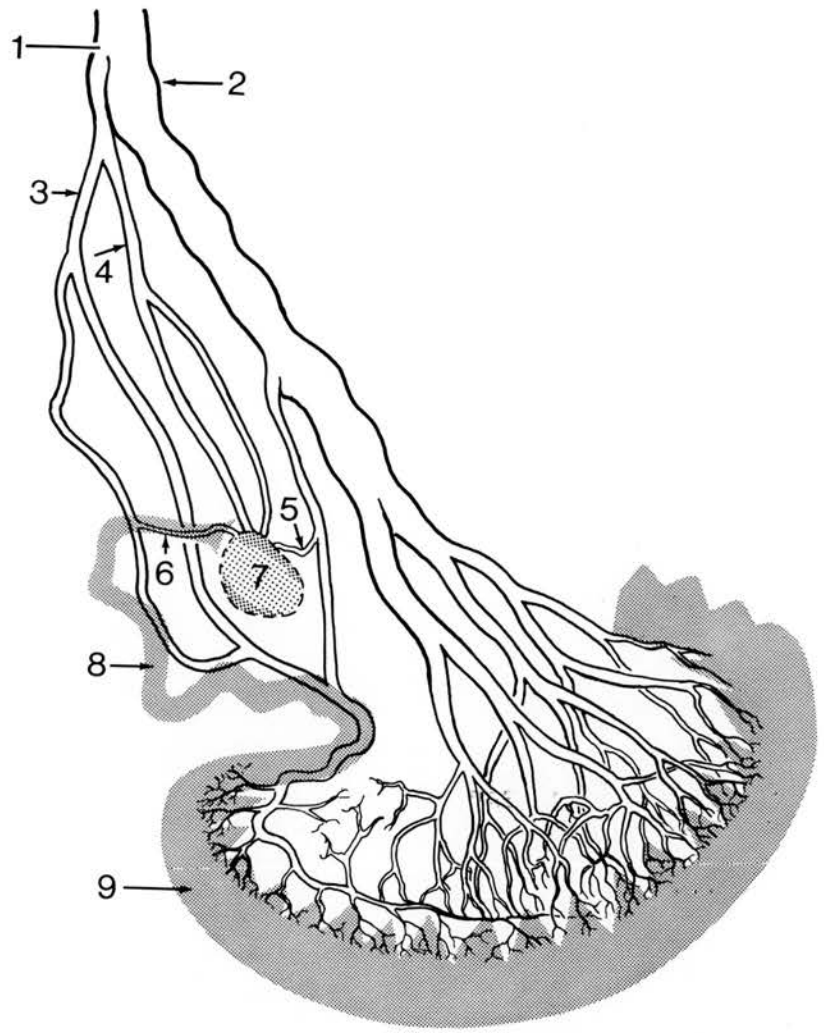
UTERO-OVARIAN VASCULATURE IN SHEEP

The vasculature of sheep uterus and ovaries has been extensively studied (Dobrowolski & Hafez, 1970; Del Campo & Ginther, 1973a, b; Ginther & Del Campo, 1973; Ginther, Del Campo & Rawlings, 1973; Del Campo & Ginther, 1974, Lee & O'Shea, 1974; 1975). The uterus and ovaries are drained through a common vein "the utero-ovarian vein". This vein and its three branches (uterine, ovarian & tubal) drain most of the uterine horn and all of the ovary and uterine tubes (Fig 2). The uterine body and cervix are drained by the uterine branch of the vaginal vein, which also anastomoses with branches of the uterine branch of utero-ovarian vein. The large veins contain tough, prominent valves. The venous drainage of the ovary and the uterine tube was found to be considerably complex. The ovarian branch of the utero-ovarian vein empties into the tubal branch in some animals and into the uterine branch in others (Del Campo & Ginther, 1973b). Rarely does the ovarian branch consist of one major vein, usually it has one or more branches, but in most animals the ovarian branch complex is very short. The venous drainage of the uterine tube is also complex and duplicates, in part, the drainage by the ovarian branch (Del Campo & Ginther, 1973b). The principal veins from the uterine tube appear to form an arch around the ovary, so that one branch of the arch enters the uterine branch of utero-ovarian vein (main uterine vein) and the other branch enters the utero-ovarian vein or joins the ovarian branch of the utero-ovarian vein (See Fig 2). Prominent anastomotic networks occur between

Figure 2

Dorsal aspect of veins of right ovary and uterine tube in a sheep (after Del Campo & Ginther, 1973b).

Ovary drained by two prominent tributaries of the ovarian branch of the utero-ovarian vein. In addition two veins from the ovary drain directly into veins from the uterine tube. Uterine tube is drained by veins which join the ovarian or uterine branches of the utero-ovarian vein. There is a prominent anastomosis of the tubal veins to a vein from the tip of the uterine horn. 1. Utero-ovarian vein; 2. uterine branch of utero-ovarian vein (main uterine vein); 3. tubal branch of utero-ovarian vein; 4. ovarian branch of utero-ovarian vein; 5. & 6. two veins from the ovary drain directly into veins from the uterine tube; 7. ovary; 8. uterine tube; 9. uterine horn.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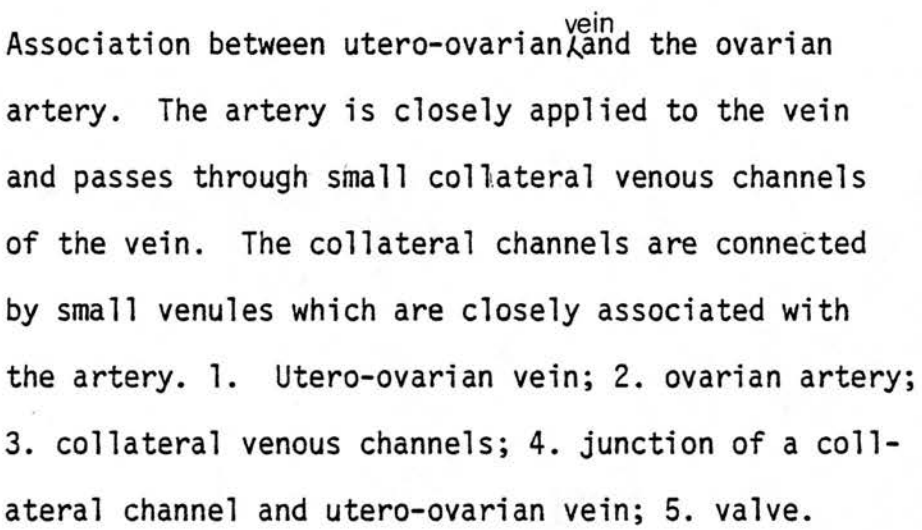
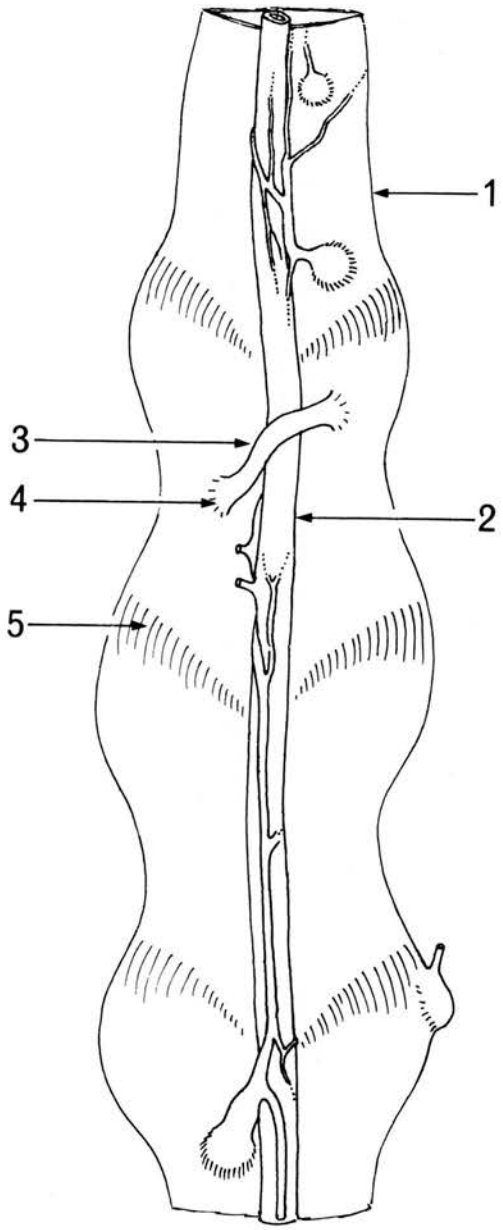
the venous systems of the left and the right sides. In the sheep this network is ventral (Del Campo & Ginther, 1973a, b) to the intercornual area, uterine body and cervix. The reproductive tract is suspended from the body wall by the broad ligaments. The uterine artery and vaginal artery on each side originate from the internal iliac artery while the ovarian arteries originate from the aorta cranial to the origin of the external iliac arteries. The utero-ovarian vein on each side terminates in the vena cava at approximately the level of the origin of the ovarian arteries. The ovarian artery follows the course of the utero-ovarian vein and is closely applied to the vein (Fig 3; and Ginther & Del Campo, 1973). This close apposition between the artery and the vein occurs over the greater part of the vein's length (approximately 12 cm). Ginther & Del Campo (1973) studied the structure of the area of close apposition between the ovarian artery and utero-ovarian vein in sheep. They found that in the initial (farthest from ovary) one-third of the main ovarian artery, small anastomotic veins connect the main uterine vein and a collateral venous network which surrounds the artery. The work of Ginther & Del Campo (1973) suggests that these areas of extensive surface contact may be the site of passage of a uterine luteolytic substance from uterine venous blood into the ovarian artery. The histological structure of the utero-ovarian vein and the closely adhering ovarian artery was studied by Del Campo & Ginther, 1974). The wall of each vessel, in the area where the two vessels were in apposition, was complete. However, the connect-

Figure 3



Association between utero-ovarian vein and the ovarian artery. The artery is closely applied to the vein and passes through small collateral venous channels of the vein. The collateral channels are connected by small venules which are closely associated with the artery. 1. Utero-ovarian vein; 2. ovarian artery; 3. collateral venous channels; 4. junction of a collateral channel and utero-ovarian vein; 5. valve.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ive tissue bundles of the adventitia in the area of apposition of the two vessels formed a single stratum so that the demarcation between vessels was poorly defined. Furthermore, the thickness of the wall of the artery at its point of contact with the vein was reduced due to reduction in the circular muscle. In the utero-ovarian vascular pedicle of sheep, the distance from lumen to lumen (thickness of the two apposed walls)was an average of 0.35 mm in histological sections while elsewhere, it was between 0.46 - 0.50 mm. In addition although venules were not found in the wall of the artery or vein, in most sections there were many venules in the connective tissue just external to the adventitia of the artery. These structural relationships between the two vessels suggest a pathway whereby the passage of a uterine luteolytic substance between the utero-ovarian vein and the ovarian artery could occur (Del Campo & Ginther, 1974; Lee & O'Shea, 1975).

The utero-ovarian arterial haemodynamics were also studied by Del Campo & Ginther (1973a). They investigated amounts of arterial blood reaching the uterus and ovaries by the uterine and ovarian branches of the ovarian artery. The ovarian artery appears to contribute to the uterine arterial supply through the uterine branch of the ovarian artery (Del Campo & Ginther, 1973a). Lamond and co-workers have done preliminary studies involving sections of the ovarian artery or its uterine branch in sheep (Lamond & Drost, 1973) and questioned the role of a veno-arterial pathway for uterine-induced luteolysis. However,

data from projects involving sectioning or ligation of arteries are difficult to interpret. It has been noted by Ginther & Del Campo (1973) that ligations of the ovarian artery in sheep are readily by-passed by the development of collateral arteries. In addition, after ligation of the ovarian artery, it seems reasonable that blood would reach the ovary through the uterine branch of the ovarian artery. Since the ovarian artery is closely applied to, and often coiled around, the main uterine vein in sheep (Ginther & Del Campo, 1973), this area could become an artificially imposed, area for a veno-arterial pathway.

THE EFFECTS OF SURGICAL ANASTOMOSIS OF UTERINE VEINS OR OVARIAN ARTERIES ON LUTEAL FUNCTIONS

The preceding experiments indicated that the main uterine vein served as the initial component of the local utero-ovarian pathway. It has been found that towards the end of oestrous cycle, sheep uterine venous blood caused luteolysis when infused into the ovarian artery by cross circulation experiments between donor sheep bearing utero-ovarian transplants and recipient sheep with ovary transplanted alone (McCracken, Baird & Goding, 1971). Ewes receiving uterine venous blood from a donor on day 15 of the oestrous cycle showed a rapid fall in progesterone secretion and a new oestrous cycle was initiated. However, the animals which received either peripheral blood from donors on day 15 or uterine blood on day 2, 6, 10 and 13 of the cycle showed only small depression of progesterone secretion rate and neither CL regression nor oestrous behaviour was induced (McCracken, Baird & Goding, 1971; McCracken, Carlson, Glew, Goding, Baird, Grēen & Samuelsson, 1972). It should be noted that some diminution in progesterone secretion rate occurred in most recipients cross circulated with donor blood early in the cycle, perhaps resulting from non-specific effects of infusing venous blood from the uterus - e.g. its lowered O_2 content or the presence of by-products from uterine metabolism (McCracken et al. 1972). These results provided good evidence that a luteolytic factor was present in uterine vein blood specifically at the time when luteal regression was taking place. Support for this concept is given by the finding that freeze-dried extracts

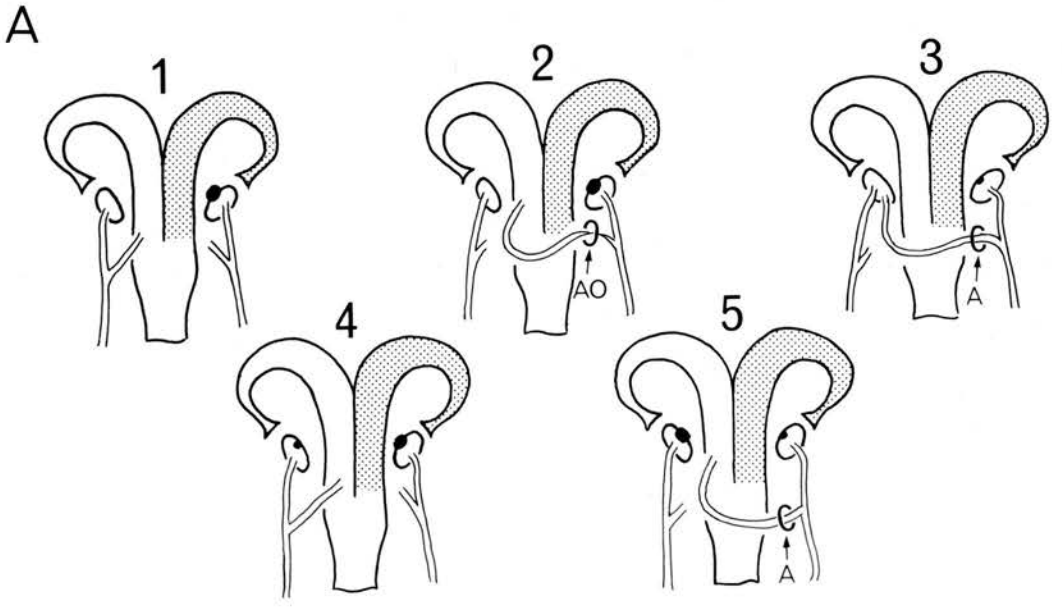
of ovine uterine vein plasma collected on day 14 of the oestrous cycle (but not on day 7 or 10) when infused into arterial supply of the sheep ovary for 6-8 hours depressed progesterone secretion by about 50%; oestrus occurred between 48 and 72 hours after the cessation of infusion (Caldwell & Moor, 1971; McCracken et al. 1972). The postulation by Barrett et al. (1971) that the uterine luteolysin could act on the CL by passing from the uterine vein directly to the ovarian artery initiated a series of experiments involving surgical anastomosis of uterine veins or ovarian arteries (Ginther, Del Campo & Rawlings, 1973; Ginther, 1974; Mapletoft & Ginther, 1975). In unilaterally hysterectomized sheep, venous blood from the uterine-intact side was diverted by surgical anastomosis of veins so that it drained into the main uterine vein on the hysterectomized side (See fig 4A, after Ginther, Del-Campo & Rawlings; 1973; Ginther, 1974; Mapletoft & Ginther, 1975). Surgical operation was done on days 7, 8 or 9 after oestrus and ewes were killed on day 20. The corpora lutea had regressed on the hysterectomized side in unilaterally ovulating ewes with a patent venous anastomosis (Fig 4A, group 3), but were maintained in ewes with an occluded venous anastomosis (Fig 4A, group 2) and in unilaterally hysterectomized ewes in which an anastomosis was not done (Fig 4A, group 1). Similar results were obtained in sheep treated with an IUD (Ginther & Bisgard, 1972). Moreover, it was found in bilaterally ovulating ewes that the CL was maintained on the uterine intact-side and regressed on the hysterectomized side in ewe without anastomosis (Fig 4A, group 4). While

Figure 4A

Diagrammatic representation of several experiments which were designed to determine the role of the main uterine vein in luteolysis at the end of the oestrous cycle in sheep (after Ginther, Del Campo & Rawlings, 1973; Ginther, 1974; Mapletoft & Ginther, 1975). A large black dot in an ovary indicates a maintained CL on day 20 and small dot indicates a regressed CL. Sheep in all groups were unilaterally hysterectomized ipsilateral to the CL (indicated by stippled area). In sheep in groups 2, 3 and 5 the main uterine vein which drained the intact horn was anastomosed to the main uterine horn on the hysterectomized side. In group 2 the anastomosis became occluded (AO). Whereas in group 3 and 5 the anastomosis remained patent (A). Sheep in groups 1, 2 and 3 were unilateral ovulators and those in group 4 and 5 were bilateral ovulators.

Figure 4B

Diagrammatic repesentation of several experiments which were designed to determine the role of the ovarian artery in luteolysis at the end of the oestrous cycle in sheep (after Ginther, Del Campo & Rawlings, 1973; Ginther, 1974; Mapletoft & Ginther 1975). A large black dot in an ovary indicates a maintained CL on day 20 and a small dot indicates a regressed CL. Sheep in group 1 were sham-operated. The remaining sheep were unilaterally hysterectomized ipsilateral (group 2 & 3) or contralateral (group 4 & 5) to the CL and anastomosis (A) of the ovarian artery was done. In group 4 & 5 the ovarian pedicle was separated (broken line) from the retained horn, separation included the uterine branch of the ovarian artery, but not the uterine branch of the utero-ovarian vein. In group 2 & 4 the uterine branch of the ovarian artery was only freed, transected and reanastomosed as a control for the anastomosis procedure.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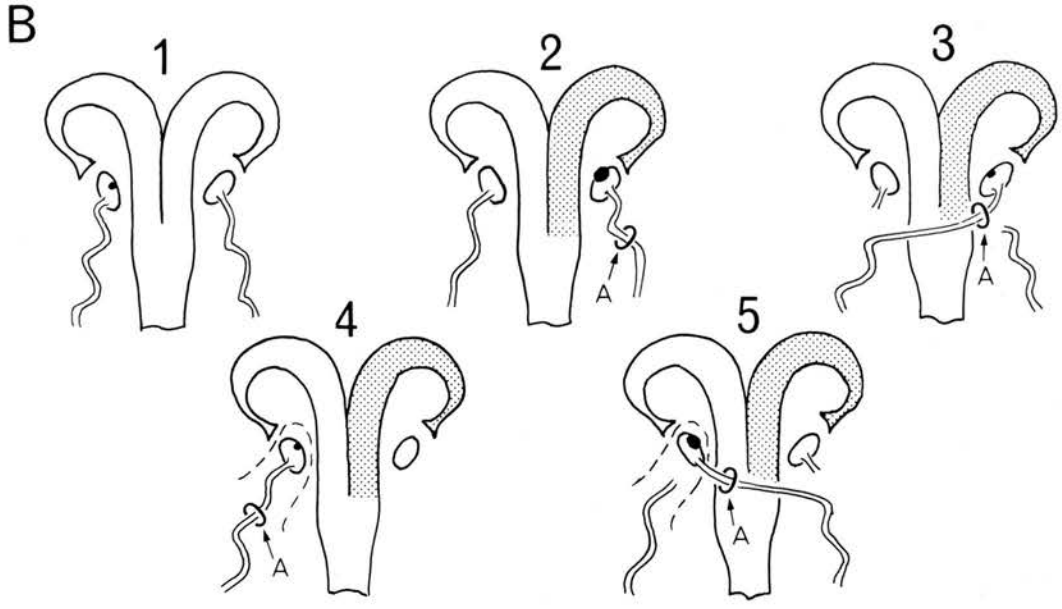
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anastomosis of the uterine-intact side to the hysterectomized side not only caused regression of the CL on the hysterectomized side but also prevented regression of the CL on the intact side (Fig 4A, group 5) (Ginther, 1974; Mapletoft & Ginther, 1975). Mapletoft & Ginther (1975) also noticed that there was a significant difference in the weight of the CL between the uterine-intact side in the control ewes and the hysterectomized side in ewe with a patent venous anastomosis. These results indicate that the main uterine vein is in itself, an adequate uterine outlet for the local transport of the uterine luteolysin. Mapletoft & Ginther (1975) also found that the CL on the uterine-intact side has regressed (100 mg) in one of the two ewes with an occluded venous anastomosis and partially regressed (294 mg) in the other, whereas the CL on the hysterectomized side were maintained in both ewes (485 and 550 mg). They concluded that occlusion of the uterine vein resulted in diversion of large quantities of uterine venous effluent through the tubal area. In this regard, Baird & Land (1973) had previously found that ligation of the main uterine vein did not prevent regression of the CL in 6 out of 10 ewes, whereas regression only occurred in 1 of 7 ewes when both the main uterine veins and the tubal veins were ligated. This regression was apparently due to the development of compensating vessels and diversion of blood through the tubal area to the ovary.

In another set of experiments in unilaterally hysterectomized sheep that had only ovulatedfrom one ovary, arterial blood prior to its entry into the ovary was diverted by surgical anastomosis.

Thus the ovarian branch of the ovarian artery on the uterus-intact side was anastomosed to that on the hysterectomized side, thus it now supplied the ovary on the hysterectomized side (see Fig 4B, after Ginther, Del Campo & Rawlings, 1973; Ginther, 1974; Mapletoft & Ginther, 1975). They found that the mean weight of the CL was less ($p<0.05$) in animals where the ovary was recieving blood from the ovarian artery on the side adjacent to an intact-uterine horn (Fig 4B, groups 1, 3 & 4) than in those animals in which the ovarian arterial blood was derived from the hysterectomized side (Fig 4B, groups 2 & 5). Thus the ovarian artery is involved in the local utero-ovarian pathway for uterine-induced luteolysis in ewes. In Mapletoft & Ginther's experiments (1975) the necessary ligation of the proximal segment of the recipients ovarian artery prevented the development of the collateral arteries to the CL-bearing ovary, thus the ovary could be supplied only through the anastomosis between the donor and recipient artery. Regression of the CL occurred in all 4 ewes after this procedure. These results indicate that the ovarian artery, is in itself an adequate ovarian inlet for the local utero-ovarian pathway without the assitance of other possible concomitant routes. This is further borne out by the finding that regression of the CL in 3 ewes and partial regression in one ewe occurred when the ovarian vascular pedicle on the uterus-intact side was isolated from the uterine horn except for the ovarian artery and the main uterine vein (Mapletoft & Ginther, 1975); these results are also consistent with the conclusion that the main uterine vein is an adequate

uterine outlet for the pathway.

Thus the results of experiments involving venous and arterial anastomosis cited above demonstrate that the local luteolytic effect of the uterus is exerted through a local veno-arterial pathway between a uterine horn and the adjacent ovary in ewes. Veins which drain the uterine horn (utero-ovarian vein and its uterine branch) serve as the proximal component and the ovarian artery and its ovarian branch as the distal component of the veno-arterial pathway. The veno-arterial pathway is an adequate pathway for uterine-induced luteolysis without the necessity of other routes between uterus and ovary.

THE STUDY OF OVARIAN FUNCTION IN THE EWE BY MEANS OF VASCULAR AUTOTRANSPLANTATION

A more direct approach to study the ovarian function is to measure the concentration of steroid hormones in the venous effluent from the ovary. However , ovarian venous blood cannot be obtained from the ovary *in situ* except as a result of surgical interference. Thus attempts have been made with varying success to obtain serial samples from individual animals by placing indwelling cannulae in the ovarian vein (Short, McDonald & Rowson, 1963; Lindner, Sass & Morris, 1964; Moore, Brown & Smyth, 1965; Mattner & Thorburn, 1969). However, in these studies the stress of cannulation on ovarian function is unknown, and the calculation of steroid secretion rates may be inaccurate in view of the difficulty in determining the blood flow from the gland. This difficulty is illustrated in the experiments of Short, McDonald & Rowson (1963) in which "minimal estimates" of the ovarian steroid secretion rates were obtained after cannulation of the major vein draining the CL or ripe Graafian follicle. They found that the blood flow rates obtained were lower in oestrous ewes than in dioestrous ones. However, Moore, Brown & Smyth (1965) using a similar technique did not find that the rate varied cyclically.

A considerable advantage would be obtained by moving the ovarian and/or the uterine vein to a more accessible location on the outer surface of the body. Thorburn & Mattner (1969, 1971) have approached this problem by anastomosing the uterine vein to the mammary vein leaving the ovaries and uterus *in situ*. The

ovaries and uterus are very inaccessible because of their location deep in the abdominal cavity. The anastomosis technique allowed regular sampling of uterine and/or ovarian blood in conscious non-pregnant (Thorburn & Mattner, 1969, 1971; Cox, Mattner & Thorburn, 1971) and pregnant ewes (Mattner & Thorburn, 1970).

The transplantation of the ovary and/or the uterus to a more accessible location in the neck has proved to be very valuable in the study of the ovarian or uterine function and the factors controlling the oestrous cycle in sheep (Goding, 1966; Goding, Harrison, Heap & Linzell, 1967; Goding, McCracken & Baird, 1967a, b; Baird, Goding, Ichikawa & McCracken, 1968; Harrison, Heap & Linzell, 1968; McCracken & Baird, 1969; McCracken, Caldwell, Tillson, Thorneycroft, & Scaramuzzi, 1969; Caldwell, 1970; McCracken, Glew & Levy, 1970; McCracken, Baird & Goding, 1971; Baird, Land, Scaramuzzi & Wheeler, 1976).

1. TRANSPLANTATION OF THE OVARY

In order to obtain exclusive long-term access to both the arterial and venous side of the ovarian circulation in the ewe a technique was evolved for autotransplantation and vascular anastomosis of the ovary and its vascular pedicle from its pelvic location to a subcutaneous site in the neck (Goding, McCracken & Baird, 1967b; McCracken & Baird, 1969). The first stage consisted of the preparation of a carotid artery-jugular

vein skin tube, with a pouch to receive the ovary and its pedicle. At least two months later, the left ovary and its pedicle was transplanted to the pouch in the neck and a patch of the aorta containing the origin of the ovarian artery was anastomosed to the left carotid artery and the utero-ovarian vein to the jugular vein. The right ovary was removed, so that total ovarian secretion entered the jugular vein in the skin loop. By occluding the jugular vein distal and proximal to the ovary, the ovarian venous blood flowed into the jugular vein between the occlusion points, where it was easily collected by means of a wide bore catheter inserted percutaneously. Blood flow was calculated by recording the time taken for a known volume, usually 25 ml of heparinized ovarian venous blood to flow freely from the jugular cannula into a graduated tube. Ovarian function was demonstrated by behavioural evidence and by measurement of the secretion of progesterone. The rate of progesterone secretion was <5 µg hr^{-1}, at oestrus and rose to approximately 200 µg hr^{-1} in the luteal phase of the cycle. This is within the range reported for the ovary in situ (Short, McDonald & Rowson, 1963). It was found that the transplantation of the ovary to a jugulo-carotid skin loop in the neck with vascular anastomosis (but leaving the uterus in situ in the abdomen) results in luteal retention (Goding, McCracken & Baird, 1967b ; Baird, Goding, Ichikawa & McCracken, 1968; McCracken & Baird, 1969; McCracken, Baird & Goding,1971).

2. TRANSPLANTATION OF THE UTERUS

The major part of the reproductive tract (the right uterine horn and oviduct, the body of the uterus and cervix, and the anterior part of the vagina) was transplanted to the neck during anoestrus in the sheep by anastomosing the right uterine artery to the left carotid artery and the right utero-ovarian vein to the left jugular vein (Goding, Harrison, Heap & Linzell, 1967). The left uterine horn was discarded leaving the utero-ovarian vein *in situ* to permit venous drainage of the left ovary. The right ovary was also discarded. The cut end of the vagina was exteriorized and sutured to the skin of the neck. The ewe with a uterine transplant had shown no oestrous behaviour 8 months after operation but had a persistently raised peripheral progesterone level. Treatment with either progesterone, pregnant mare's serum gondotrophin (PMSG) or luteinizing hormone (LH) was without effect. At autopsy 31 days after PMSG injection, two large functional corpora lutea were found in the abdominal ovary. These experiments showed that transplantation of the uterus with vascular anastomoses to the neck but leaving one ovary in the abdomen results in luteal retention (Goding, Harrison, Heap & Linzell, 1967).

Autotransplantation of the uterus into the omentum or peritonium had no apparent effect on oestrous cycles in sheep. Corpora lutea formed and regressed normally after severing all direct utero-ovarian connections (Zhordania & Gotsiridze, 1963; Niswender, Kaltenbach & Dziuk, 1967; Niswender, Dziuk, Graber &

Kaltenbach, 1970; Niswender 1968). However, when endometrium was grafted into the flank of hysterectomized ewes the corpora lutea were still maintained signficantly longer than the normal cycle. Luteal regression occurred after about 30-40 days in these animals (Caldwell, Rowson, Moor & Hay, 1969). Moreover, endometrial tissue inserted into corpora lutea at the time of hysterectomy failed to hasten the return to oestrus, although histological examination of the ovaries showed that a local and graded pattern of cellular degeneration had occurred around the grafted endometrial tissue (Rowson, Moor, Hay & Caldwell, 1969).

3. TRANSPLANTATION OF THE OVARY AND UTERUS

In an attempt to obtain normal ovarian function after transplantation to the neck of the sheep, Harrison, Heap & Linzell (1968) transplanted the left ovary and left uterine horn together with the body of the uterus and cervix, and anterior vagina. The uterine artery and a patch of aorta with the origin of the ovarian arterywere anastomosed end-to-side to the left carotid artery and the utero-ovarian vein was anastomosed end-to-side to the left jugular vein. The vagina was fistulated through a hole in the skin at the base of the neck and the uterine body and horn were lightly anchored to the neck muscles. The right horn of the uterus and the right ovary were removed. Blood samples were collected from the utero-ovarian vein via the left jugular vein. Oestrus recurred at regular intervals. It was found that the secretion rate of prog-

esterone was low on day 2 of the cycle (<0.06 μg min^{-1}) and showed a significant increase (up to 3.5 μg min^{-1}) during the luteal phase, i.e. a mean of 1.4 ± 0.5 μg min^{-1} between day 5 and 16. These values are similar to those reported previously by Short, McDonald & Rowson (1963) for the abdominal ovary of the sheep in the luteal phase. It was concluded that the transplantation to the neck of the ovary with its adjacent uterine horn as a block of tissue results in regular luteal regression and cycles of normal length (Harrison, Heap & Linzell, 1968; McCracken, Caldwell, Tillson, Thorneycroft & Scaramuzzi, 1969; McCracken, Glew & Levy, 1970; Baird, Land, Scaramuzzi & Wheeler, 1976).

It is clear that the majority of transplantation experiments of McCracken's group support the view that the anatomical relationship between the ovary and uterus is very important. However, the result of uterine transplantation to the omentum, peritonium or flank (p.32) suggest that the uterus also has a systemic effect, since it is difficult to visualize any other pathway for the transplanted uterine tissue to exert control over the functional life-span of the corpora lutea in these cases (Caldwell, 1970). As uterine transplantation for the most part successfully reduces the prolonged luteal life-span following hysterectomy, it was natural to attempt to control the CL function by the use of extracts of uterine tissue.

The Effects of Uterine Extracts and Homogenates on Luteal Function

The preceding results strongly suggest that in the sheep, the non-pregnant uterus has the ability to cause luteolysis. The obvious corollary is a "uterine luteolytic hormone". Numerous workers have attempted to prepare an active extract with the objective of eventual purification and identification of the luteolytic principle.

In studies on sheep to this end, *in vitro* systems have been successful whereas *in vivo* ones have failed. Thus ewes hysterectomized on day 12, 13 or 14 of the oestrous cycle which received uterine extracts which have been lyophilized to dryness did not show reduced CL weights at slaughter (Kiracofe & Spies, 1964). Similarly, Kiracofe and co-workers (Kiracofe, Spies & Gier, 1963; Kiracofe, 1966; Kiracofe, Menzies, Gier & Spies, 1966) also reported that injections of ethersoluble extracts or lyophilized homogenates of uteri from ewes at various stages of the oestrous cycle did not effect corpora lutea weight or histology of hysterectomized or pregnant ewes. Caldwell and co-workers (Caldwell, Rowson, Moor & Hay, 1969) used endometrium stripped from the uterus of sheep at various stages of the oestrous cycle and then homogenized, freeze-dried and powdered. About 10 to 12 mg of this material was compressed into pellets and inserted directly into sheep corpora lutea following hysterectomy. None of the animals tested in this way returned to oestrus and histological examinations of the ovarian tissue at

various intervals showed no luteal degeneration. However, Caldwell, Moor & Lawson (1968) were more successful. Endometrium extracts from the uterus of the sheep at various stages of the oestrous cycle were injected intraperitoneally on days 9 and 10 of hysterectomized pseudopregnant hamsters. Significant shortening of the life-span of the corpora lutea occurred in nine of the sixteen hamsters when the extracts were prepared from feeze-dried endometrium from sheep on days 14 or 15. However extracts prepared from other days in the cycle havelittle effect on the length of the pseudopregnancy of the hysterectomized hamsters (Caldwell, Moor & Lawson, 1968). The interesting finding of the above group that uterine extracts from specific times of the oestrous cycle may be effective in inducing luteal regression in hysterectomized hamster, certainly added to the growing weight of evidence in support of a "uterine luteolytic factor". Stormshak & Kelley (1967) observed that slices of sheep intercaruncular endometrium obtained at day 8 and day 14 of the oestrous cycle when incubated with luteal tissue in vitro resulted in a significant ($p<0.01$) increase in steroidogenesis. However, there was no significant differences due to age of the luteal tissue or the age of endometrium.

Channing (1966; 1969) has shown that granulosa cells can be grown in tissue culture after harvesting them from intact graafian follicles of human and horses. In tissue culture these granulosa cells behave like luteal tissue and even secrete progesterone; such a tissue culture system seems to be ideally suited for tests for luteolytic activity in uterine extracts. Schomberg

(1967), using sow granulosa cells growing as a monolayer in culture under conditions similar to those described by Channing (1966), reported that uterine flushings from day 12 to 18 had a lytic effect, while flushing from all other days in the procine oestrous cycle had no effect on the granulosa cultures. With slight modifications in technique Caldwell, Rowson, Moor & Hay (1969) showed that when sheep granulosa cells were cultured and the freeze-dried endometrial preparations were tested for potential "luteolytic" activity, 66% of the day 14 and 15 extracts were deleterious to the cells while only 25% of the extracts prepared from other days in the cycle were. To test the specificity of the active element in the "lytic" extracts Caldwell, Rowson, Moor & Hay (1969) applied the same medium to fibroblast monolayer cultures. They found that only an extract that had no effect on the granulosa cells was without any influence on the fibroblasts. Likewise Schomberg (1969a, b) reported that procine uterus flushings which were "lytic" to pig granulosa cells also killed kidney cell monolayer cultures after 24 hr. The resistance of the various cultures to these flushings varied considerably. It was found that sheep trophoblast and pig kidney cells were more resistant than sheep granulosa cells which in turn were more resistant than pig granulosa cells (Schomberg, 1969b).

Further attempts by Caldwell, Rowson, Moor & Hay (1969) to characterize the active luteolytic factor in the sheep endometrial extracts showed that the substances were nondialysable and heat stable at 50^{o}C for 30 min. The fractionation pattern on a Sephadex G-25 column suggested that the most active fraction was of a low molecular weight (below 1500). However, both the higher and lower

molecular weight fractions showed some deleterious effects of granulosa culture. Diluting the various fractions by 3:1 appeared to eliminate the activity of most of these fractions, however the most active molecular weight band was very "lytic" even at this dilution (Caldwell, Rowson, Moor & Hay, 1969).

THE EFFECTS OF PITUITARY GLAND ON LUTEAL FUNCTION IN THE SHEEP

The formation of the CL and its subsequent secretory activity in the non-pregnant sheep are the result of the trophic action of a number of pituitary hormones; while during gestation there is an additional feto-placental trophic influence (see Denamur, 1974). While discussing the luteotrophic effects of the pituitary, one must not forget the effects of the uterine factors (McCracken, Carlson, Glew, Goding, Baird, Gréen & Samuelsson, 1972; Goding 1973; 1974). The secretory activity of the CL is the net result of an interplay between these trophic and lytic factors; thus the maintenance, regression or stimulation of the CL results from a change in balance between these trophic and lytic effects.

There is general agreement between both the French (Denamur, Martinet & Short, 1966a, b) and the American (Kaltenbach, Graber, Niswender & Nalbandov, 1968a) workers in this field, that pituitary support is essential for the continued maintenance of functional corpora lutea during the latter half of the ovine oestrous cycle. Three basic methods have been used to investigate the luteotrophic effect of the pituitary, namely, hypophysectomy, stalk section and administration of exogenous hormones. These will each be considered in turn:-

HYPOPHYSECTOMY

One of the earliest contributions to our understanding of the role of the pituitary in luteal function was made by Denamur &

Mauléon (1963a). They used the morphological development of the CL as an index of luteal function. They reported that hypophysectomy of prepubertal ewe-lambs soon after ovulation (induced with PMSG and HCG) was without effect on the histological structure and life-span of the corpora lutea up to day 12, i.e. about 10 days after hypophysectomy. Hypophysectomy of adult cycling ewes between days 3 and 4 also allowed histologically normal luteal development until days 10 to 12 (Denamur, Martinet & Short, 1966a). However, measurement of progesterone concentrations in these animals showed that it remained normal for only about 5 days after hypophysectomy. When hypophysectomy was performed at day 10, structural regression and decline in progesterone secretion were more rapid. These results show that the effects of hypophysectomy are very dependent upon the age of the CL, and that pituitary secretions are necessary for the attainment of normal progesterone secretion in the second third of the cycle.

Denamur & Mauléon (1963b) also investigated the effect of hysterectomy coupled with hypophysectomy on luteal function. Hysterectomy prolongs the life-span of the ovine CL (Wiltbank & Casida, 1956; see also p.14-15) and the French workers confirmed this observation. However, when hypophysectomy and hysterectomy were carried out together on the day of ovulation, the corpora lutea regressed about 20 days later. But if hypophysectomy was delayed until 20 days after hysterectomy the corpora lutea regressed completely within 6 days (Denamur & Mauléon, 1963b). A marked

fall in the progesterone concentration in ovarian vein blood and luteal tissue occurred within 48 hr of hypophysectomy (Deanmur, Martinet & Short, 1966a, b). They concluded from these experiments that sheep CL have a predetermined 15 days life-span, and this can be increased only by the intervention of the pituitary. This is borne out by the finding (Mauléon & Denamur, 1966) that injection of anterior pituitary extracts (1 mg/day) into sheep hypophysectomized and hysterectomized at ovulation had allowed complete maintenance of the corpora lutea 20 days later. Kaltenbach & coworkers (Kaltenbach, 1968; Kaltenbach, Graber, Niswender & Nalbandov, 1968a), reported that when both normal cyclic ewes and anoestrous ewes in which ovulation had been induced by PMSG & HCG were hypophysectomized on the day of ovulation (day 1) and the CL were examined on day 8, there was no difference in the response to hypophysectomy. They conclude that pituitary support is indeed necessary for the normal formation of the corpora lutea in the ewe. In other experiments they (Kaltenbach, Niswender, Graber & Nalbandov, 1966; Kaltenbach, 1968; Kaltenbach, et al, 1968a) hypophysectomized cyclic and anoestrous animals 5 days after indication of ovulation and autopsied them on day 12. They found that complete regression of the corpora lutea was present by day 12 in all animals in which there was no pituitary tissue remaining in the sella turcica and 6 out of 12 incompletely hypophysectomized animals. A highly significant reduction in luteal weight, progesterone content and concentration was observed in all hypophsectomized animals when compared with the appropriate day 12-controls. The corpora lutea of

hypophysectomized animals were also significantly lighter than the corpora lutea removed from the day 5 control animals, indicating that hypophysectomy induced active luteal regression. Thus pituitary hormones appear necessary not only for the formation of CL but also for the continued maintenance of luteal structure and function throughout the oestrous cycle. So it seems that the results of the French (Denamur, 1968) and the American (Kaltenbach, 1968) workers differ only in that the former maintain that the CL will form, at least in part, after hyophysectomy on day 1, whereas the latter contend that hypophysectomy completely inhibits the CL formation. By comparing progesterone secretion rate in the presence and absence of the pituitary, Hixon & Clegg (1969)found that hypophysectomy of mature reproductively active ewes at mid-cycle resulted in a highly significant 12-fold decrease in the secretion rate of progesterone within 5 hr. This decrease was composed of a 3-fold drop in ovarian venous progesterone concentration and a 4-fold decrease in the rate of ovarian blood flow. More recently, Denamur, Martinet & Short, (1973) have reinvestigated the problem by paying particular attention to the technique of hypophysectomy. Even after removal of the entire pituitary from the sella turcica, a significant amount of pars tuberalis tissue may remain attached to the pituitary stalk and the base of the brain; this may have some effect on the life-span of the CL (Denamur, 1968; Hixon & Clegg, 1969) and LH secretion (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969; Niswender, Reichert, Midgley & Nalbandov, 1969). After establishing the detailed time course of events leading to luteal regression in hysterectomized and hypophysect-

omized ewes, Denamur, Martinet & Short (1973) used them as test animals to investigate the luteotrophic activity of purified ovine pituitary gonadotrophins (see later).

STALK SECTION

Denamur, Martinet & Short (1966a) carried out pituitary stalk sections in ewes to investigate the part played by the pituitary gland in the control of the CL. They found that regardless of the time of stalk section, the CL continued secreting progesterone in concentrations similar to those in normal animals, while pituitary stalk sections following hysterectomy at mid-cycle allowed the corpora lutea to continue secreting large amounts of progesterone for at least 15 days after the operation. These experiments emphasise that pituitary stalk section does not produce the same effect as hypophysectomy. The effects of chlorpromazine(a drug that depresses hypothalamic function) treatment (Coudert & Short, 1968) are similar to those of stalk section. Moreover, stalk section seems to allow the severed pituitary gland to continue secreting a luteotrophin at least for a time and support the view that the cyclical corpora lutea of the sheep is under a dual control. There is a pituitary luteotrophin, whose secretion continues after stalk secetion, and a uterine luteolysin which is dominant to the luteotrophic stimulus and can still function normally after pituitary stalk section. Although the fact that the CL of the hysterectomized animal cannot function for more than about 15 days after stalk section suggest that the luteotrophic stimulus may be complex and involving more than one hormone (Denamur, Martinet & Short, 1966a; Thibault, 1966).

LUTEOTROPHIC FACTORS IN THE NON-PREGNANT SHEEP

Both the French (Denamur, Martinet & Short, 1966a) and the American workers (Kaltenbach, Graber, Niswender & Nalbandov, 1968a) as well as Hixon & Clegg (1969) agree that the luteotrophins of importance in the ewe are LH and/or Prolactin. Since hypophysectomy in the ewe during the luteal phase of the cycle results in regression of the CL (see p.39 and also Nalbandov & Cook, 1968; Denamur, Martinet & Short, 1973) and whether prolactin is necessary for maintenance of normal luteal function (Denamur, Martinet & Short, 1966a, 1973; Rothchild, 1966; Denamur, 1974) or not (Karsch, Cook, Ellicott, Foster, Jackson & Nalbandov, 1971), there is general agreement that LH is an essential component of the luteotrophic complex.

LUTEINIZING HORMONE (LH)

The results of hypophysectomy point to the necessity for continued luteotrophic support for normal function of CL. Numerous recent reports concern the trophic influence of pituitary gonadotrophins on maintenance and secretory activity of corpora lutea of sheep (Domański, Skrzeczkowski, Stupnicka, Fitko & Dobrowolski, 1967; Denamur, 1968, Kaltenbach, Graber, Niswender & Nalbandov, 1968b, Hixon & Clegg, 1969; Fuller & Hansel, 1970, McCracken, Baird & Goding, 1971). All of these authors concluded that LH plays a major luteotrophic role in sheep. The first report of a stimulatory action of a known gonadotrophin on in vitro production of progesterone was by Legault-Démare, Mauléon & Suarez-Soto (1960). They reported that

when PMSG was added to incubating CL-slices, there was an increase in the amount of progesterone produced. These studies have been criticizedby Savard, Marsh & Rice (1965) but gonadotrophins have since been found to increase the rate of *in vitro* progesterone sythesis by CL-slices in many animals (see Armstrong, 1968). For instance in sheep LH has been shown to increase steroid synthesis when added to luteal tissue incubated *in vitro* (Kaltenbach, Cook, Niswender & Nalbandov, 1967). Furthermore, Domański *et al* (1967) have indicated that LH is capable of stimulating the secretory activity of the CL *in vivo*, thus the perfusion of the ovary *in situ* with ovine LH through a side branch of ovarian artery on day 8 of the oestrous cycle resulted in an increased secretion rate of progesterone within 20 to 30 min. When the perfusion was terminated the rate of progesterone secretion quickly reverted to the original level. A similar result was obtained by Cook, Kaltenbach, Niswender, Norton & Nalbandov (1969), only the increase in progesterone secretion was less. Changes in progesterone secretion in response to LH in ewes bearing an ovarian transplant are small, temporary and variable (Baird, Collett & Land, 1971; Collett, Land & Baird, 1973; Land, Collett & Baird, 1974). In hypophysectomized ewes, Kaltenbach *et al*. (1968a, b) found that continuous infusion of LH maintained CL life-span, but interruption of the infusion for a short time resulted in irreversible luteolysis. However, Kaltenbach *et al*. (1968b) showed that crude LH alone is luteotrophic in hypophysectomized non-pregnant or pregnant sheep while purified LH is only partially luteotrophic in that it prevents complete degeneration of the CL, but can maintain pregnancy for only a limited time. In contrast FSH, prolactin or oestrogen alone show

no luteotrophic properties in hypophysectomized animals. They suggest that the reason crude LH maintained CL in these experiments lies in the fact that is was infused constantly. They estimated that much less than 1 μg of LH hr^{-1} actually reached the CL (Kaltenbach et al. 1968b). Similarly Hixon & Clegg (1969) show that intrajugular injection of ewes hypophysectomized at mid-cycle with 50 mg LH resulted in significant increase in ovarian venous progesterone concentration within 10 to 20 min. Moreover, subcutaneous injection of anti-bovine LH serum into ewes on day 2 to 6 of the oestrous cycle resulted in a signficant reduction in weights and progesterone concentration of corpora lutea by day 9. However, the animals did not show behavioural oestrus until 28 and 29 days later (Fuller & Hansel, 1970). These findings suggest that LH is a luteotrophic factor in the sheep and the delay in return to oestrus suggests that it may also be necessary for follicular development and oestrogen production. However, it should be noted that both genetic and seasonal variation in the reproductive activity of sheep have been shown to be reflected in the level of LH (Land, Pelletier, Thimonier & Mauléon, 1973; Land, Wheeler & Carr, 1976; Legan, Karsch & Foster, 1977; Yuthasastrakosol, Palmer & Howland, 1977; Land, Carr & Thompson, 1979).

Since CL persists in the absence of the uterus it may be reasoned that adequate luteotrophic support is available and under normal circumstances the luteolytic effect of the uterus overcomes the luteotrophic action of pituitary hormones (Short,

1969). The possibility of increasing the concentration of luteotrophin to overcome the luteolytic effect of the uterus was investigated by Karsch and co-workers (Karsch, Noveroske, Roche & Nalbandov, 1969a; Karsch, Roche, Noveroske, Foster, Norton & Nalbandov, 1971). They found that continuous intravenous infusion of 2.5 mg of purified ovine LH into intact normal ewes starting on day 10-12 after heat prolonged the life-span of corpora lutea. The weight and progesterone concentrations of the maintained corpora lutea indicated that they were as active as corpora lutea from non-treated ewes on day 9-13 of the oestrous cycle. Continuous infusion was less effective when began on day 13-14 after heat and totally ineffective when initiated on day 15. Thus it appears that increased levels of luteotrophin cannot save the CL once luteolysis has been initiated. In another study, these workers (Karsch, Noveroske, Roche & Nalbandov, 1969b; Karsch, Noveroske, Roche, Norton & Nalbandov, 1970) found that destruction of follicles by X-irridiation of ovaries increased the effectiveness of continuous infusion of purified LH in prolonging the life-span of the CL of the intact ewe. When follicles were present the amount of infused LH required to maintain corpora lutea was 4 times greater than that needed when the follicles had been destroyed. This may indicate the necessity for intact follicles to be present for the production of an uterine luteolytic substance. Thus LH or hormones with LH activity can overcome the luteolytic effects of the uterus during the oestrous cycle and the luteolytic mechanisms can be modified by luteotrophic hormones. The luteolytic consequences of an IUD inserted

on day 4 of the cycle (Stormshak & Hawk, 1966; Stormshak, Lehmann & Hawk, 1967) or injection of oestradiol on day 11 and 12 (Akbar, Rowe & Stormshak, 1971; Bolt, Kelley & Hawk, 1971; Kann & Denamur, 1973) or injections of progesterone (Lewis, Taylor & Inskeep, 1968) in intact sheep, are prevented by the administration of large quantities of HCG or LH. Thus it appears that normally the uterine luteolysin is secreted in sufficient quantities to dominate the effects of the luteotrophin complex. If, however, the level of luteotrophin especially LH is increased experimentally (Ginther, Hawk & Casida, 1966; Karsch, Roche, Noveroske, Foster, Norton & Nalbandov, 1971) the uterus is ineffective in causing regression of CL.

PROLACTIN

The concentration of prolactin in the plasma has been measured throughout the oestrous cycle (Reeves, Arimura & Schally, 1970; Bryant, Greenwood, Kann, Martinet & Denamur, 1971; Davis, Reichert & Niswender, 1971; Kann, 1971; Cumming, Brown, Goding, Bryant & Greenwood, 1972). These authors show that the level of prolactin increased at oestrus very near to the time of the ovulatory release of LH, and in some instances copulation appeared to result in an additional surge of prolactin production (Cumming, Brown, Goding, Bryant & Greenwood, 1972). Apparently the secretion of progesterone during the oestrous cycle is not continually dependent on the concentration of prolactin or LH, even though

both hormones are capable of stimulating progesterone output (Domański, Skrzeczkowski, Stupnicka, Fitko & Dobrowolski, 1967; Hixon & Clegg, 1969).

In another study (McCracken, Uno, Goding, Ichikawa & Baird, 1969) investigated in vivo effects of gonadotrophins by infusing prolactin and FSH in a dose of 0.1 - 100 $\mu g\ hr^{-1}$ directly through an autotransplanted ovary. They found that both prolactin and FSH had no effect on either blood flow or progesterone secretion. The results of Karsch, Cook, Ellicott, Foster, Jackson & Nalbandov (1971) showed that the exogenous infusion of LH (2.5 mg day^{-1}) started on day 12 to 20 of oestrous cycle may maintain corpora lutea in the presence of the uterus whereas infusion of prolactin (2.5 or 5.0 mg day^{-1}) cannot do so. Likewise the addition of prolactin to incubated slices of sheep CL also had no effect on progesterone synthesis in vitro (Kaltenbach, Cook, Niswender & Nalbandov, 1966; 1967; McCracken, Baird & Goding, 1971).

The luteotrophic properties of prolactin have been demonstrated by injection of prolactin into hypophysectomized ewes (Denamur, 1974). Prolactin treatment increased the weight of the corpora lutea, and caused an increase in the progesterone concentration in the ovarian venous blood. Moreover prolactin was shown to retain its luteotrophic activity after heating to $100^{o}C$ at pH7 for several minutes, a treatment which would destroy any contaminating LH (Denamur & Short, 1973). Thus prolactin alone seems to have some luteo-

trophic action. However the adminstration of 0.25 to 0.5 mg day^{-1} LH along with prolactin to hypophysectomized sheep resulted in corpora lutea whose weight, RNA content and progesterone secretion rate were at least equal to those of hysterectomized controls (Denamur & Short, 1973). It is interesting to contrast this luteotrophic effect of prolactin to its inability to prolong the life-span of the CL in the hypophysectomized-hysterectomized sheep (Denamur & Mauléon, 1963b; Denamur, 1968; Kaltenbach, Graber, Niswender & Nalbandov, 1968b). Moreover, the secretion of prolactin can be reduced to less than 1.5 mg ml^{-1} in the ewes injected with the ergot derivative, Ergocryptin (CB-154) without altering the normal cyclic behaviour in intact animals (Niswender, 1972, Denamur, 1974; Kann & Denamur, 1974).

The consequences of pituitary stalk section also favour a luteotrophic role for prolactin (Denamur, Martinet & Short, 1966a, 1970). These authors postulated that the maintenance of the secretory ability of the CL after stalk section must be due in part to the continued release of pituitary prolactin. Measurements of prolactin during the normal oestrous cycle of the sheep and after section of the pituitary stalk were carried out to verify this hypothesis (Bryant, Greenwood, Kann, Martinet & Denamur, 1971). Since there is very marked reduction in the size of the hypophsis after stalk section (Adams, Daniel & Pritchard, 1963), the small amount of prolactin found supported the suggestion that the continuation of the CL function after stalk section, is due to persistence of prolactin secretion (Bryant, Greenwood, Kann, Martinet & Denamur, 1971). However, this suggests that prolactin may have

some part to play in the maintenance of the CL following hysterectomy. To this end, hysterectomized-hyphyophysectomized animals were used in a series of experiments to test the luteotrophic properties of sheep pituitary gonadotrophins (Denamur, 1968; Denamur, Martinent & Short, 1973; Denamur & Short, 1973). Denamur and co-workers this time hypophysectomized sheep by an improved technique that allowed removal of the pituitary stalk and most of the pars tuberalis. They found that doses of FSH & LH alone or as mixture were unable to prevent complete luteal regression while, prolactin was able to maintain functional CL for 12 days, although at a considerably reduced level of activity. Moreover, a mixture of prolactin and small amounts of LH were able to maintain the CL at a level of activity comparable to that seen in hysterectomized animals before hypophysectomy. These results indicated that prolactin and LH are both necessary for the maintenance of the sheep CL and that these two hormones together make up the "luteotrophic complex".

GROWTH HORMONE (GH)

As adenocorticotrophic hormone (ACTH) along with either prolactin or GH can maintain milk secretion in the goat (Cowie, 1966), there appears to be a close functional relationship between GH and prolactin. Early attempts to measure growth hormone by radioimmunoassay were reported in human by Utiger, Parker & Daughaday (1962). Since this first report, a number of workers have measured and identified the properties of GH in other animals. In

sheep, many studies have been carried out to measure GH concentrations in plasma (Machlin, Takahashi, Hornio, Hertelendy, Gordon & Kipnis, 1968; Wallace & Bassett, 1970. Hertelendy, Todd, Ehrhart & Blute, 1972; Chamley, Jonas & Parr, 1976). However as Kaltenbach, Cook, Niswender & Nalbandov (1966) failed to stimulate progesterone sythesis in the sheep corpora lutea by GH, there is no evidence to suggest that GH plays any role in the luteotrophic mechanism of the pituitary gland.

FOLLICLE-STIMULATING HORMONE (FSH)

The plasma concentrations of FSH have recently been measured by radioimmunoassay during the ovine oestrous cycle (Kerdelhué, Kann & Jutisz, 1972; L'Hermite, Niswender, Reichert & Midgley, 1972; Hopkinson & Pant, 1973; Salamonsen, Jonas, Buger, Buckmaster, Chamley, Cumming, Findlay & Goding, 1973; Pant, Hopkinson & Fitzpatrick, 1977) and found to be about 100-160 ng ml^{-1} during the cycle but 180-220 ng ml^{-1} on the day of oestrus (L'Hermite *et al.* 1972). McCracken, Uno, Goding, Ichikawa & Baird (1969) infused purified ovine pituitary preparations through an autotransplanted ovary but found that FSH and prolactin in doses of 0.1 - 100 µg hr^{-1} had no effect on either blood flow or steroid secretion. Moreover it was found that the addition of FSH and prolactin to incubated ovine luteal tissue *in vitro* had no effect on progesterone synthesis (Kaltenbach, Cook, Niswender & Nalbandov, 1967; McCracken, Baird & Goding, 1971). On the other hand progesterone synthesis in ovine corpora lutea was found to be stimulated by LH or HCG

but not by FSH (Kaltenbach, Cook, Niswender & Nalbandov, 1966). Forbes & Clegg (1969) determined the plasma level of FSH by radioimmunoassay techniques following insertion of an IUD into the anterior portion of both uterine horns of ewes. They found that plasma levels of FSH fluctuated greatly but there was no signficant difference between IUD-containing and control ewes. Intramuscular or continuous intravenous administration of FSH in doses of 0.5, 1.0 and 5.0 mg per day was unable to prevent complete luteal regression in the hypophysectomized - hysterectomized sheep (Denamur, Martinet & Short, 1973). This was true regardless of whether or not the pars tuberalis tissue was removed (Denamur, Martinet & Short, 1973; Denamur & Short, 1973). It thus appears that FSH plays no role in the luteotrophic mechanism of the pituitary.

THE EFFECTS OF PROGESTERONE & OESTRADIOL ON LUTEAL FUNCTION

It is well established in the sheep that the life-span of the CL can be modified experimentally by administration of exogenous steroids. The interpretation of these treatments is complicated by the fact that steroids may act at various sites. They may have a direct action on the CL, or an effect on the pituitary gland/hypothalamus to modify the release of luteotrophic hormones, or act directly on the uterine endometrium to effect the production of luteolytic substance (s). There is evidence that steroids can act on all three of the above sites to affect luteal function.

OESTRADIOL

The plasma concentrations of oestradiol throughout the oestrous cycle in sheep has now been measured by many groups (Norman, Eleftheriou, Spies & Hoppe, 1968; Moore, Barrett, Brown, Schindler, Smith & Smyth, 1969; Scaramuzzi, Caldwell & Moor, 1970; Cox, Mattner & Thorburn, 1971; Obst, Seamark & Brown, 1971; Bjersing, Hay, Kann, Moor, Naftolin, Scaramuzzi, Short & Younglai, 1972; Holst, Braden & Mattner, 1972; Mattner & Braden, 1972; Cox, Thorburn, Currie, Restall & Schneider, 1973; Barcikowski, Carlson, Wilson & McCracken, 1974; Yuthasastrakosol, Palmer & Howland, 1975; Pant, Hopkinson & Fitzpatrick, 1977).

Moore et al. (1969) showed that oestradiol-17β first appeared on day 15 and its concentration rose rapidly during the immediate pre-oestrous period to reach a peak concentration of over 1.0 ng ml^{-1} plasma 20 to 30 hr before the onset of oestrus. It then declined rapidly to reach almost non-detectable concentrations by 24 hr after the onset of oestrus. Scaramuzzi et al. (1970) measured oestrogen in ovarian venous blood by radioimmunoassay and also found that the concentration of oestradiol-17β had started to increase by day 15, in addition they found two oestrogen peaks on day 3 to 5 and 7 to 10. The peak on day 4 was confirmed (Cox, Mattner & Thorburn, 1971; Obst, Seamark & Brown, 1971; Mattner & Braden, 1972), while the day 8 peak has been detected in peripheral plasma by Obst, Seamark & Brown (1971). The three surges of oestradiol secretion do not always occur from the same ovary and are not necessarily associated with the ovary containing the CL or the ovary from which the next ovulation will occur (Mattner & Braden, 1972). Serum oestradiol concentrations are low on days 11 and 12, begin to rise on day 13 or 14 and continue to increase until day 15 or 16. This increase precedes the decline in serum progesterone level which occurs about day 14 to 15 (Moore et al. 1969 ; Scaramuzzi et al. 1970; Bjersing et al. 1972; Denamur & Short, 1973; Warren, Hawk & Bolt, 1973). This increase in serum oestradiol during the critical period coincides with the end of the first wave of follicular growth (Brand & de Jong, 1973) and the beginning of the enlargement of the follicles for the next oestrus (Smeaton & Robertson, 1971; Holst, Braden & Mattner, 1972). Microscopical examination of the ovaries indicated

that high oestradiol-17β secretion was from ovaries containing follicles of 3.5 to 4.5 mm in diameter; which may be at an early stage of atresia and not likely to ovulate (Holst, Braden & Mattner, 1972). Moreover various workers (Karsch et al. 1969b; 1970; Ginther, 1971; Hixon, Gengenbach & Hansel, 1975) have shown that destruction of follicles by X-irradiation or electro-cautery delay CL regression. These findings indicate that the ovarian follicles and presumable oestradiol play a role in the regression of the CL in normal ewes at the end of oestrous cycle.

The effect of administration of exogenous oestradiol at various times of the oestrous cycle of the sheep throws some light on this conclusion. It was found that daily injection of oestradiol benzoate (1 mg per day) from the beginning of the oestrous cycle was able to prolong the life-span of the CL for several weeks (Denamur & Mauléon, 1963b; Foote, 1964; Piper & Foote, 1965; 1967; 1968; 1970; Denamur, Martinet & Short, 1970; Kann & Denamur, 1973). The maintained corpora lutea were found to have normal weight and contain relatively normal amounts of RNA and DNA, when compared to corpora lutea of normal cycling ewes on day 12 (Denamur, Martinet & Short, 1970). They were also actively secreting progesterone (Piper & Foote, 1970; Denamur, Martinet & Short, 1970).

As already mentioned, the pituitary gland continues to secrete luteotrophic hormones for a limited time even when the pituitary stalk has been sectioned, but even so the CL regress

at the expected time if the uterus is left *in situ* (Denamur, Martinet & Short, 1966a; 1970). However, it was found that injection of oestradiol to such stalk-sectioned ewes resulted in luteal maintenance for at least 20 days without any major increase in gonadotrophin secretion (Denamur, Martinet & Short, 1970; Denamur & Kann, 1973; Kann & Denamur, 1973). However, the administration of oestradiol is without any luteotrophic effect in hypophysectomized sheep (Denamur & Mauléon, 1963b, Kaltenbach, Graber, Niswender & Nalbandov, 1968b; Denamur, Martinet & Short, 1970; Denamur & Kann, 1973), and it also has no luteotrophic effect when implanted directly into the CL (R.M. Moor in Denamur & Short, 1973). These results indicate that a direct action of the oestradiol on the CL does not occur.

The effect of oestradiol on the pituitary luteotrophin secretion has also been studied by Kann & Denamur (1973). They found that in intact ewes, oestradiol given from day 3 resulted in luteal maintenance associated with a significant increase in the secretion of LH and prolactin. However, injection of prolactin alone, even in very large amounts cannot maintain the life-span and the secretory activity of the CL in either the intact or hypophysectomized sheep (Denamur, 1968; Kaltenbach *et al.* 1968b; Denamur & Short, 1973). Oestrogen administration has been found to be followed by a large surge of LH (Brown, Catt, Cumming, Goding, Kaltenbach & Mole, 1969; Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969; Pelletier & Signoret, 1969; Radford, Wheatley & Wallace, 1969; Radford, Wallace & Wheatley,

1970), and the results of Radford, Wheatley & Wallace (1969) provide strong support for the concept that the pre-ovulatory release of LH in ewe is a result of oestrogenic stimulation. However, as a rise in LH can follow progesterone withdrawal without any rise in oestradiol-17β , it appears that in the intact ewe the LH peak at oestrus may be a response to both progesterone withdrawal and oestrogenic stimulation (Radford, Wheatley & Wallace, 1969). This possibility is also supported by the finding that oestradiol injection at the end of progesterone priming in ovariectomized ewes resulted in an LH surge similar to that at oestrus (Scaramuzzi, Tilsson, Thorneycroft & Caldwell, 1971). Moreover, pretreatment of anoestrous ewes with PMSG or oestradiol before a single injection of synthetic LH - releasing hormone (LH-RH) significantly increased the release of LH compared to that after injection of LH-RH alone. (Haresign & Lamming, 1978). Although the above findings favour the involvement of LH in the luteotrophic effect of oestrogen, Karsch, Noveroske, Roche & Nalbandov (1969a) reported that continual intrajugular infusion of purified LH can maintain the lifespan of CL to day 20 in normal ewes while a lower dose of LH will maintain the CL only in ewes whose follicles have been destroyed by X-irridation (Karsch, Noveroske, Roche & Nalbandov, 1969b). Likewise, Goding, Blockey, Brown, Catt, Cumming(1970) showed that the base-line values of LH release were depressed by continuous adminstration of oestradiol. Thus it seems unlikely that the luteotrophic effect of the oestradiol is mediated by pituitary

LH, especially since oestrogen injections have a luteotrophic effect in pituitary stalk-sectioned animals, when LH secretion is presumably abolished.

A luteotrophic effect of oestradiol via FSH is equally unlikely. Treatment of anoestrous ewes with either oestradiol-17β or gonadotrophin-releasing hormone (GnRH) was found to be followed by coincident release of FSH and LH (Jonas, Salamonsen, Burger, Chamley, Cumming, Findlay & Goding, 1973). However, progesterone injection to such animals can suppress the FSH surge following oestradiol-17β but failed to effect the FSH surge induced by GnRH (Cumming, Baxter, Buckmaster, Jonas, Findlay & Goding, 1974). This indicates a positive feedback of oestradiol on FSH release which can only occur in the absence of progesterone. However contrary to this is the finding that continuous injection of oestradiol-17β immediately following ovariectomy holds FSH levels below those found in ovariectomized ewes; the basal FSH level is higher after ovariectomy than in the entire ewe (Salamonsen, Jonas, Burger, Buckmaster, Chamley, Cumming, Findlay & Goding, 1973; Cumming et al. 1974). From these and other experiments it appears that the baseline levels of FSH are regulated by a negative feedback system of oestradiol-17β. However, this feedback seems to be slow in action as it takes 2-3 weeks for FSH levels to become maximal after ovariectomy (Cumming et al. 1974). Thus although oestradiol can induce the release of GnRH which in turn releases FSH (Salamonsen et al. 1973) there is no evidence that FSH is luteo-

trophic in sheep (see p.52).

The possibility that oestrogen is luteotrophic by a direct action on the uterus by interfering with the normal luteolytic mechanism is suggested by the persistence of the luteotrophic effect following pituitary stalk section (Denamur, Martinet & Short, 1970; Denamur & Kann, 1973; Kann & Denamur, 1973). It is interesting to note here that the embryo may maintain the life-span of the CL by a similar action on the uterus (Moor & Rowson, 1966c, d; Moor, 1968).

In contrast to the luteotrophic effects of oestrogen injected at the beginning of the oestrous cycle in sheep, it was found that injection of oestradiol from about day 9 to 12 induced premature regression of corpora lutea (Howland, Kirkpatrick, Woody, Pope & Casida, 1968; Stormshak, Kelley & Hawk, 1969; Hawk & Bolt, 1970; Piper & Foote, 1970; Akbar, Rowe & Stormshak, 1970; Denamur & Kann, 1973; Warren, Hawk & Bolt, 1973). The weights and progesterone content of the CL of the treated ewes were signficantly reduced compared to those of controls. However, the injection of oestradiol on day 11 and 12 failed to cause regression of the CL in hysterectomized ewes (Stormshak, Kelley & Hawk, 1969; Akbar, Rowe & Stormshak, 1970; Bolt & Hawk, 1972; Denamur & Kann, 1973; Bolt & Hawk, 1975). The weights of corpora lutea in both ovaries were reduced by oestradiol administration to unilaterally-hysterectomized ewes, but the corpora lutea in the ovary adjacent to intact uterine horn were significantly smaller in size than those in the opposite ovary (Akbar, Rowe & Stormshak, 1971). This suggests that, part at least of the effect

of oestradiol on the CL was mediated through the adjacent uterine horn. This lytic effect of oestrogen persists after pituitary stalk section suggesting that the hormone is not acting centrally on the hypothalamus (Denamur & Kann, 1973). Thus it seems that the luteolytic properties of oestradiol like its trophic effects reflect a direct action of this hormone on the uterus.

Many studies (Warren, Hawk & Bolt, 1973; Ford, Weems, Pitts, Pexton, Butcher & Inskeep, 1975, Louis, Parry, Robinson, Thorburn, & Challis, 1977) suggest that a period of progestational influence facilitates the luteolytic effect of oestradiol and the rise of endogenous oestradiol is the stimulus which acts on the progesterone-primed uterus to cause the production of a luteolytic substance at the end of the oestrous cycle. This is supported by the finding that the increase in oestradiol secretion by the developing follicle at the end of the oestrous cycle seems to precede the decline in progesterone secretion of the CL (Moore et al.,1969; Scaramuzzi et al., 1970; Bjersing et al., 1972; Denamur & Short, 1973; Warren, Hawk & Bolt, 1973). Also several groups of workers (Ford, Pexton, Wilson, Butcher & Inskeep, 1973; Barcikowski, Carlson, Wilson & McCracken, 1974; Ford, Weems, Pitts, Pexton, Butcher & Inskeep, 1975) have now found that oestradiol administration on day 10, 11, 12 of the cycle results in increased concentrations of the luteolytic hormone in the uterine venous blood (see later).

PROGESTERONE

Several investigators have reported the concentrations of progesterone in peripheral or ovarian venous plasma of sheep during the oestrous cycle (Neher & Zarrow, 1954; Edgar & Ronaldson, 1958; Short, 1958; 1961; Short, McDonald & Rowson, 1963; Stormshak, Inskeep, Lynn, Pope & Casida, 1963; Plotka & Erb, 1967; Smith & Robinson, 1969; Stabenfeldt, Holt & Ewing, 1969; Thorburn, Bassett & Smith, 1969; Plotka, Erb & Harrington, 1970; Bjersing et al., 1972; McNatty, Revfeim & Young, 1973; Sarda, Robertson & Smeaton, 1973; Bedford, Harrison & Heap, 1974; Ythasastrakosol, Palmer & Howland, 1975; Quirke & Gosling, 1976; Pant, Hopkinson & Fitzpatrick, 1977; Quirke, Hanrahan & Gosling, 1979). Progesterone concentrations were found to be lowest during oestrus and for 2 days after oestrus (about 0.25 ng/ml) increasing to about 1.6 ng/ml on day 5 to 6 and reaching a peak of about 3.70 ng/ml between day 8 and 14. This was followed by a decline over the 36 hr preceding the next oestrus. Some variation was observed in the rate of decline of plasma progesterone between individual animals (Bassett, Oxborrow, Smith & Thorburn, 1969; Fylling, 1970; Pant, Hopkinson & Fitzpatrick, 1977).and breed difference in ovulation rates have been shown to be reflected in the levels of plasma progesterone (Land, Pelletier, Thimonier & Maulĕon, 1973; Quirke & Gosling, 1976; Wheeler & Land, 1977; Quirke, Hanrahan & Gosling, 1979). Progesterone has also been determined in luteal tissue of ewe (Stormshak, Inskeep, Lynn, Pope & Casida 1963; Smith & Robinson, 1969). A good relationship between concentration in the ovarian venous blood and that in luteal tissue in the oestrous cycle was reported by Deane,

Hay, Moor, Rowson & Short (1966). Moreover, positive correlations were found between ovarian venous plasma progesterone and the weight, diameter, total progesterone content and concentration of the CL (Smith & Robinson, 1969). The weight of the CL, its progesterone content and the concentration of progesterone in the peripheral plasma were found to increase in the post-ovulatory phase then remain relatively constant until about the 14th day of the cycle before declining at the time of luteolysis (Plotka, Erb & Harrington, 1970; Geschwind, 1972). A positive association has also been found between the peripheral plasma concentrations and the number of corpora lutea in the ovaries after stimulating with PMSG in the ewe. (Gordon, 1958; Short, 1960; Thorburn, Bassett & Smith, 1969; Bindon, Ch'ang & Turner, 1971; Eastwood, Payne, Fairclough & McDonald, 1976). However, progesterone concentrations have been found to be of little value in distinguishing between ewes with one or two CL (Edgar & Ronaldson, 1958; Bindon, Ch'ang & Turner, 1971; Robertson & Sarda, 1971; Lamond, Gaddy & Kennedy, 1972; Quirke, Hanrahan & Gosling, 1979).

The effects of exogenous progesterone in ewes has been extensively studied by a number of workers. Initial studies on the use of progesterone were employing it as an agent for the synchronisation of oestrous cycles (Dutt & Casida, 1948; O'Mary, Pope & Casida, 1950). Administration of progesterone to sheep early in the oestrous cycle shortened the length of that cycle (Zimbelman, Pope & Casida, 1959; Woody, First & Pope, 1967; Ginther, 1968b; Lewis, Taylor & Inskeep, 1968; Woody, Ginther

& Pope, 1968; Ginther, 1969; Thwaites, 1971; Hecker, Bray & Wodzicka-Tomaszewska, 1974), and caused early regression of the CL (Ginther, 1968b; Smith & Robinson, 1969). However, Woody, Ginther & Pope (1966a, 1968) and Woody (1968) found that hysterectomized ewes treated with progesterone from day 1 to 10 had failed to return to oestrus by day 60 indicating that hysterectomy reduces the inhibitory action of progesterone and suggesting that the uterus may play a role in the action of progesterone on the CL. Moreover, Ginther, (1968b) injected progesterone into unilaterally hysterectomized sheep that had one CL in each ovary on days 2 to 5 of the oestrous cycle. By day 15 he found that the average CL weight on the side of the retained horn was signficantly less then that on the side of the removed horn. These results not only suggest that the effect of progesterone may be mediated through the uterus but also that it is through the local relationship between the uterine horn and the CL in the ipsilateral ovary. The effects of exogenous progesterone depends on the stage of the cycle in which the treatment begun. Ginther (1968b) and Lewis, Taylor & Inskeep (1968) showed that oestrous cycle length was significantly shortened by the injection of progesterone for less than 6 days when treatment was started on day 1, 2, 3 or 4 but not on day 5; while injection of progesterone on day 13 to 16 extended the cycle beyond the time of expected oestrus but did not significantly alter the mean weight of the CL by day 17 (Ginther, 1969). It was found that corpora lutea induced in anoestrous ewes were smaller if the ewe was pretreated with progesterone prior to ovulation than if no

progesterone was given (Woody, Ginther & Pope, 1966b; 1967; Woody, 1968). The life-span of the corpora lutea induced in such ewes was relatively short and depended upon the amount of progesterone acting at the time they were formed (Inskeep et al., 1963, 1964a, b; Moor, Booth & Rowson, 1966). The data of Ginther (1968b) suggest that complete regression of the CL does not occur until some time after progesterone treatment and the CL taken at day 15 from ewes treated with progesterone on day 1 to 4 were still only half as large as CL of the controls. Furthermore, Warren, Hawk & Bolt (1973) reported apparently normal CL at day 10 in ewes treated with progesterone on day 2 to 5. This suggests that progesterone injection early in the cycle does not induce immediate luteolysis, but allows the uterus to become luteolytic earlier than during normal oestrous cycle. As progesterone concentrations are high between day 5 and 13 but initiation of the uterine luteolytic mechanism does not occur until about day 12, it has been assumed that this 8 days period is required for the uterus to develop sufficient sensitivity to enable it to respond to an endogenous stimulus which initiates the luteolytic function of the uterus. The decline in the progesterone after this period is evidently coupled with a rise in oestradiol and the changes in the levels of the two hormones is responsible for the induction of behavioural oestrus (McCracken, Baird & Goding, 1971).

In addition, it was found that the combined action of oestradiol and progesterone is necessary for the secretion of sufficient LH during the oestrous cycle (Goodman, 1978; Karsch & Legan,

1978). However, the patterns of LH and progesterone secretion during the oestrous cycle suggest that progesterone may inhibit tonic LH secretion (Hauger, Karsch & Foster, 1977). Karsch and his co-workers (Karsch, Legan, Hauger & Foster, 1977; Goodman & Kasrsch, 1979) reinvestigated the possibility that progesterone might govern tonic LH secretion in the ewe by examining the ability of physiological increments in circulating progesterone to inhibit the tonic LH secretion. They found that administration of progesterone by means of silastic implants into ovariectomized ewes signficantly reduced the LH concentration in the serum. In other ewes in which the treatment was initiated immediately following ovariectomy they found that the physiological increments in circulating progesterone prevented the typical post-castration rise in serum LH concentrations and maintained circulating LH at mid-luteal phase levels. Furthermore, in long-term ovariectomized ewes in which serum LH concentrations had been suppressed by chronic treatment with oestradiol implants during the anoestrous season, physiological increments in circulating progesterone were found to be effective in maintaining low mide-luteal phase LH concentrations when oestradiol implants were removed. These findings lead to the conclusion that progesterone can exert potent inhibition of tonic LH secretion in the ewe; for the full effect it requires the action of another hormone, probably oestadiol (Karsch, Legan, Hanger & Foster, 1977).

From this data it appears that both progesterone and oestrogen are required for the synthesis and release of the luteolytic hormone

However, progesterone does not cause production of the luteolytic substance directly but can act on the uterus to cause premature sensitivity to the stimulus which initiates the release of the luteolytic substance. The requirement of a prior period of progesterone influence on the uterus before oestradiol effects the release of the luteolytic hormone suggests that progesterone might provide the condition necessary for its synthesis. As a result of these events the cycle-shortening effect of exogenous progesterone can be explained on the basis of premature activation of the uterine luteolytic hormones which operate at the end of the oestrous cycle. The action of progesterone and oestrogen on the release and inhibition of pituitary gonadotrophins does not appear to be directly related to the action of these steroids on luteal life-span in the non-pregnant animal.

PROSTAGLANDINS AND LUTEAL FUNCTION IN SHEEP

LUTEOLYTIC EFFECTS OF PROSTAGLANDIN $F_2\alpha$ IN SHEEP

As previously shown, evidence accumulated indicating the existence of uterine luteolytic substance in sheep. However the early attempts to isolate a luteolytic substance from the sheep endometrium or entire uterus were mainly unsuccessful (Kiracofe, Menzies, Gier & Spies, 1966). It was not until 1969, that Pharriss & Wyngarden first demonstrated the luteolytic properties of $PGF_2\alpha$ and showed that the injection of this hormone markedly shortened the length of pseuodopregnancy in rats. Since these first studies extensive research has been carried out in sheep to demonstrate the luteolytic properties of $PGF_2\alpha$. However, there is now a great deal of evidence to suggest that $PGF_2\alpha$ is indeed the luteolytic hormone in sheep. Independently, McCracken's group (McCracken, Glew & Scaramuzzi, 1970) and Goding's group (Barrett, Blockey, Brown, Cumming, Goding, Mole & Obst, 1971; Chamley, Buckmaster, Cain, Cerini, Cerini, Cumming & Goding, 1972) found that infusion of $PGF_2\alpha$ to a sheep with ovarian transplants resulted in premature luteal regression. The initial doses of $PGF_2\alpha$ used by McCracken, Glew & Scaramuzzi (1970) were 50 or 100 $\mu g\ hr^{-1}$ for 6 hours which decreased progesterone secretion to less than 5% of the pretreated rate within 24 hr after initiation of the infusion. However, Chamley, Buckmaster, Cain, Cerini, Cerini, Cumming & Goding (1972); and Cerini, Cain, Chamley, Cumming, Findlay & Goding (1973) showed that infusion of $PGF_2\alpha$ in a dose of 40 $\mu g\ hr^{-1}$ for 4 hr or 10 $\mu g\ hr^{-1}$ for 7 hr caused a decrease in the progesterone secretion to 50% of the

control. Moreover, they found that infusion of $PGF_2\alpha$ even at a dose of 2 μg hr^{-1} for 9 hr decreased progesterone gradually and some animals returned to oestrus (Chamley, Brown, Cain, Cerini, Cerini, Cumming, Goding & Kragt, 1972; Chamley, Buckmaster, Cain, Cerini, Cerini, Cumming & Goding, 1972). In conscious intact ewes, Thorburn & Nicol (1971) were able to show that infusion of 40 μg hr^{-1} or 10 μg hr^{-1} for 3 hr of $PGF_2\alpha$ into a branch of ovarian artery caused regression of CL and the animals returned to oestrus in 60 hr. A similar effect was also noticed after infusion of 20 to 40 μg hr^{-1} of $PGF_2\alpha$ for 4-8 hr into a branch of the uterine vein, on the same side as the CL-bearing ovary (Goding, Baird, Cumming & McCracken, 1971; Thorburn & Nicol, 1971). Moreover, Goding and co-workers (Goding, Cain, Cerini, Cerini, Chamley & Cumming, 1972; Chamley, Cerini, Cerini, Cumming, Goding & O'Shea, 1974) and Hearnshaw, Restall & Gleeson (1973) showed that $PGF_2\alpha$ infusion after day 8 of the oestrous cycle into the uterine vein, ipsilateral to the ovary bearing a CL reduced progesterone secretion to low levels. No effect was noticed,when $PGF_2\alpha$ at a dose rate of 200 μg hr^{-1} or 500 μg hr^{-1} for 3 hr was infused by a systemic route (Chamley et al. 1974). Furthermore, the injection of $PGF_2\alpha$ directly into a large follicle in the ovary containing the CL was shown to be one of the most effective routes in producing complete luteal regression (Inskeep, Smutny, Butcher & Pexton, 1975; Pratt, Butcher & Inskeep, 1975; Fogwell, Lewis, Butcher & Inskeep, 1977). These experiments clearly suggest that the luteolytic effect of $PGF_2\alpha$ is through a local pathway. Douglas & Ginther (1973) further showed that injection of either 6 or 8 mg of $PGF_2\alpha$ by intramuscular

(I.M.) or intrauterine (I.U.) routes on day 8 significantly shortened the mean length of the oestrous cycle and significantly decreased the weight of the corpora lutea. Moreover, the average CL weight was significantly less in ewes given 2 mg $PGF_2\alpha$ I.U. than in ewes given 2 mg of $PGF_2\alpha$ I.M. Nett, McClellan & Niswender (1976) also found that I.U. administration of $PGF_2\alpha$ to ewes on day 9 or 10 of the oestrous cycle resulted in premature regression of the CL and reduced levels of progesterone in the systemic circulation. In addition these workers also found a reduction in the ovarian blood flow to the ovary containing the CL. Active immunization of the ewe against $PGF_2\alpha$ extended the oestrous cycle (Scaramuzzi, Baird, Wheeler & Land, 1973).

From all these studies, it is clear that $PGF_2\alpha$ is a potent luteolytic substance in sheep and its luteolytic effect was more pronounced when given locally (i.e. by intrauterine routes) than when given systemically. Furthermore, it was found (Binder, Bowler, Brown, Crossley, Hutton, Senior, Slater, Wilkinson & Wright, 1974; Baird & Scaramuzzi, 1976) that a synthetic analogue of $PGF_2\alpha$ (16-aryloxy prostaglandin $F_2\alpha$, ICI 80 996) induce luteal regression when injected systemically (the effective dose is 100-fold less than that of $PGF_2\alpha$). Acritopoulou, Haresign & Lamming, (1978) successfully used this analogue of $PGF_2\alpha$ to synchronize ovulation in sheep.

Some other prostaglandins, for example $PGF_1\alpha$, also have luteolytic properties when infused to the arterial supply of transplanted ovary in sheep (Aldrige, Barrett, Brown, Funder, Goding, Kaltenbach, Mole, 1970; Carlson, Rugg, Glew, Barcikowski & McCracken, 1972). However, both $PGF_1\alpha$ and arachidonic acid failed to show a luteolytic

effect in sheep when injected into a large follicle in the CL-containing ovary (Inskeep, Smutny, Butcher & Pexton, 1975). Neither PGE_1 or PGE_2 show luteolytic effects in sheep (Aldridge et al. 1970; Carlson et al. 1972; Inskeep et al. 1975; Henderson, Scaramuzzi & Baird, 1977; Mapletoft, Miller & Ginther, 1977). In fact PGE_2 was found to counteract the luteolytic effects of $PGF_2\alpha$ when both hormones were given by perivascular injection to non-pregnant sheep (Henderson et al. 1977; Mapletoft et al. 1977). Even so PGE_2 has been identified in homogenates of endometrium from non-pregnant ewes (Wilson, Butcher & Inskeep, 1972; Lewis, Jenkins, Fogwell & Inskeep, 1978).

RELEASE OF PROSTAGLANDINS FROM THE UTERUS OF THE SHEEP

Since the first discovery of prostaglandin in human seminal fluid (Goldblatt, 1933, 1935; von Euler, 1934, 1935, 1936; von Euler & Hammarstöm, 1937; Eliasson, 1959), extensive work has been carried out which demonstrate that most human and animal tissues contain extractable amounts of prostaglandins or prostaglandin-like material (see Karim, 1972 for references). Moreover many workers have now studied the secretion of prostaglandins in the uterine venous blood and their presence in the endometrium of sheep. The first report was that of Bland, Horton & Poyser (1971), who identified and measured $PGF_{2\alpha}$ in the uterine venous blood of sheep by gas-liquid chromatography /mass spectometry. They found a concentration of up to 8 ng ml^{-1} of $PGF_{2\alpha}$ at the end of the oestrous cycle when the CL is starting to regress. Almost simultaneously and using a similar method of extraction Gréen, Samuelsson, Carlson & McCracken (1971) reported the presence of $PGF_{2\alpha}$ in the uterine venous blood in a higher concentration (25 ng ml^{-1} of blood) at the time of luteal regression (day 15) than that earlier on day 6 (2 ng ml^{-1}). In addition Wilson, Cenedella, Butcher & Inskeep (1972) examined the uterine endometrial tissue in sheep on day 3, 5, 11 and 14 for $PGF_{2\alpha}$ and found maximal concentration on day 14. The concentrations of $PGF_{2\alpha}$ at day 14 were significantly higher than on all other days. In a subsequent study $PGF_{2\alpha}$ concentrations in endometrial tissue were found to increase steadily from day 12 to 16 of the oestrous cycle while the levels

of $PGF_{1\alpha}$ and PGE_2 remained low or undetectable (Wilson, Cenedella, Butcher & Inskeep, 1971; Wilson, Butcher, Cenedella & Inskeep, 1972).

The endotoxin from Salmonella enteritidis (Gärtner) Castellani & Chambers has been found to increase the endometrial level of prostaglandin four-fold when injected into pregnant mice (Skarnes & Harper, 1972). It is possible that prostaglandin released from the uterus in response to bacterial infection may influence luteal function. Greenwood and Kerry (1975) found that the inflammatory fluid, taken from a mild aseptic inflammatory lesion, induced by implanting a Teflon chamber subcutaneously into a sheep, contained prostaglandins. Also prostaglandins have been detected in pyometrial fluid in the cow, bitch and ferret (Heap & Poyser, 1975). Inflammatory fluid obtained from the uterus appears to contain $PGF_2\alpha$ predominantly, unlike inflammatory fluid from other sites which contains mainly prostaglandins of the E series (Greenwood & Kerry, 1975; Heap & Poyser, 1975). However, pyometra in the cow is often associated with prolonged luteal function despite the accumulation of $PGF_2\alpha$ in the pyometrial fluid contained within the uterine lumen (Heap & Poyser, 1975). In sheep with one ovary transplanted to the neck, Harrison, Heap, Horton & Poyser (1972) found the accumulation of large volumes of sterile fluid (up to 1900 ml). This fluid contained a high concentration (up to 4000 ng ml^{-1}) of $PGF_2\alpha$ but no prostaglandins of the E series. In such sheep, the cyclic ovarian activity ceased due to the persistence of the CL in the transplanted ovary. Plasma progesterone concentrations similar to those of the luteal phase of the normal oestrous cycle

of the sheep were maintained (Goding, Harrison, Heap & Linzell, 1967). Uterine fluid in pregnancy also contained high concentrations of $PGF_2\alpha$ (up to 1500 ng ml^{-1}) (Harrison, Heap & Poyser, 1976). Progesterone is implicated in this $PGF_2\alpha$-rich accumulation of uterine fluid as similar fluid with concentration of $PGF_2\alpha$>200 ng ml^{-1} occurred in the non-pregnant ewe treated with progesterone for more than 100 days (Amoroso, Harrison, Heap & Poyser, 1973; Harrison, Heap & Poyser, 1976).

Further support for the release of $PGF_2\alpha$ from the uterus have been provided by the studies of the effect of the IUDs. The insertion of an IUDs between days 2 and 4 of the oestrous cycle resulted in increased uterine venous plasma and endometrial concentrations of $PGF_2\alpha$ when measured 3 to 4 days later (Spilman & Duby, 1972; Wilson, Cenedella, Butcher & Inskeep, 1972; Pexton, Ford, Wilson, Butcher & Inskeep, 1973). Moreover, Wilson, Cenedella, Butcher & Inskeep, (1972) found a significant increase in levels of $PGF_2\alpha$ in ewes receiving an IUD in each uterine horn on day 2 of the cycle and killed on day 5. However, the rapid regression of the CL in sheep after the insertion of an IUD adjacent to the CL early in the oestrous cycle (Ginther, Pope & Casida, 1966) implies that the IUD stimulates synthesis and release of a uterine luteolytic hormone. The shortening effect of the IUD on the oestrous cycle has been shown to be overcome by the treatment of Indomethacin, an inhibitor of prostaglandin synthesis (Spilman & Duby, 1972). Indomethacin has also been found to inhibit luteolysis in unoperated cycling ewe (Lewis & Warren, 1975). These findings indicated that the luteolytic effect of an IUD can be explained satisfactorily by premature release of uterine $PGF_2\alpha$. The concentrations of $PGF_2\alpha$

found between day 2 and 4 of the cycle in the uterine venous plasma and endometrium of sheep bearing an IUD were as high as those found at the end of a normal oestrous cycle. Corpora lutea size, their progesterone content and concentration and the peripheral plasma level of progesterone were correspondingly greatly reduced (Spilman & Duby, 1972; Pexton, Ford, Wilson, Butcher & Inskeep, 1975). The ability of an IUD in the contralateral horn to cause luteolysis when inserted at day 2, but not when inserted on day 5 (Ginther, 1968b, 1974; see p.8) was attributed to higher concentrations of the luteolytic hormone in the uterine vein of the opposite side, following insertion on day 2. The higher concentrations may be due to greater production of luteolysin and/or greater crossover of venous blood at day 2. This latter interpretation was supported by the finding that $PGF_{2\alpha}$ was present in high concentration in the main uterine vein on both sides when an IUD was inserted into only one uterine horn during dioestrus (Pexton, Ford, Wilson, Butcher & Inskeep, 1973).

Thorburn and co-workers (Thorburn, Cox, Currie, Restall & Schneider, 1972, 1973; Cox, Thorburn, Currie, Restall & Schneider, 1973) studied the secretion of PGF in the ewe during the oestrous cycle by insertion of catheters into the utero-ovarian veins. Using radioimmunoassay and with frequency sampling they detect a complex series of PGF peaks between day 13 to 16 of the cycle. These peaks were of short duration and increased in frequency as oestrus approached. Very low concentrations were detected at other times. The early PGF peaks at day 13 coincide with early luteal

regression and a transient marked decrease in progesterone followed each PGF peak until luteolysis was complete. Sampling only twice daily through an indwelling catheter introduced via the saphenous vein, Fitzpatrick & Sharma (1973) found that blood from the posterior vena cava anterior to the junction with uterine veins contained low concentrations of PGF until 4 days prior to oestrus when it increased significantly (1260 to 2705 pg ml^{-1}). Also, Baird, Land, Scaramuzzi & Wheeler (1976) measured $PGF_2\alpha$ in utero-ovarian venous blood collected from ewe with utero-ovarian transplants. They found that the first significant rise of $PGF_2\alpha$ secretion occurred on day 12-14 at the time of decline of progesterone secretion, although the episodic release of $PGF_2\alpha$ was maximal (10 ng ml^{-1}) on the day before the onset of oestrus. Furthermore, Lewis, Fogwell, Thayne & Inskeep (1976) showed that the concentrations of PGF were similar in the plasma of uterine veins draining all parts of the uterus on day 6 to 8 but by day 15 of the cycle, some parts of the uterus were secreting more PGF than others.

Louis, Parry, Robinson, Thorburn & Challis (1977) reported that the uterine caruncular tissue (composed of epithelium, connective tissue and blood vessels) always contained more PGF than the intercaruncular area(composed of endometrium and myometrium) and released more PGF and 13, 14-dihydro-15-oxo $PGF_2\alpha$ during incubation in vitro. However, Pexton, Ford, Wilson, Butcher & Inskeep (1975) found that concentrations of PGF were similar in caruncular and intercaruncular tissue but both were considerably higher than in the myometrial tissue. Moreover, both endometrium and myometrium were found to release $PGF_2\alpha$ during incubation in vitro, but oxytocin

can enhance this release from endometrial tissue only (Roberts, McCracken, Gavangan & Soloff, 1976).

From all these data, it can be concluded that $PGF_2\alpha$ is released from the uterus into the uterine vein in increased amounts just prior to luteal regression in sheep. However, it was necessary to establish that these levels of $PGF_2\alpha$ were sufficeint to cause luteolysis. For this reason Thorburn & Nicol (1971) infused $PGF_2\alpha$ into a branch of the uterine vein of ewes between day 6 and 9 of the cycle at a rate of 40 $\mu g\ hr^{-1}$ for 6 hr. Such an infusion gave a concentration of 10 $ng\ ml^{-1}$ of $PGF_2\alpha$ in the uterine venous blood which is the range of the peak concentration found at day 13 of the normal cycle. Peripheral plasma progesterone concentration fell to a low level and the animals returned to oestrus in 60 hr. This indicated that endogenous level of $PGF_2\alpha$ in the uterine cycle are quite enough to initiate luteal regression. Other workers (McCracken, Glew & Scaramuzzi 1970; Barrett, Blockey, Brown, Cumming, Goding, Mole & Obst, 1971) found that infusion of $PGF_2\alpha$ in doses equivalent to those found in the uterine veous blood on day 15, into the ovarian artery on day 6 of the oestrous cycle failed to cause luteal regression but did cause only a slight decrease in plasma progesterone. The administration of similar amounts of $PGF_2\alpha$ I.M. or via the jugular vein failed to have any luteolytic effect (Thorburn & Nicol, 1971; Douglas & Ginther, 1973; Chamley, Cerini, Cerini, Cumming, Goding & O'Shea, 1974). This failure of systemic infusion of $PGF_2\alpha$ to cause regression of CL can be attributed partly to a dilution effect and

partly to the known ability of the lungs to rapidly metabolize $PGF_2\alpha$ (Ferreira & Vane, 1967; Piper, Vane & Wyllie, 1970; Piper & Vane, 1971). This also accounts for the necessity of a local pathway for the transfer of the luteolytic hormone from the uterine vein to the adjacent ovary.

TRANSFER OF PROSTAGLANDIN $F_2\alpha$ FROM UTERUS TO OVARY IN SHEEP

As mentioned previously (p. 21 & Fig. 3), it is clear in sheep that the ovarian artery followed the course of the utero-ovarian vein and is closely applied to it. Furthermore, selective ligation experiments (p. 28) of the vascular connections between the uterus and the ovary did indeed indicate that a vascular pathway was essential for normal luteal regresssion. Hansel (1969) suggested that a substance might diffuse from the vein into artery and thus pass directly to the ovary. McCracken, Baird & Goding (1971) suggested the possibility of the existence of a counter-current mechanism whereby the uterine luteolysin could reach the ovary. The counter-current mechanism was investigated by infusion of H^3-labelled $PGF_2\alpha$ into the uterine vein and measuring the amount of radioactivity in the ovarian artery at the hilus of the ovary and in the iliac artery as a control. The amount of radioactivity associated with $PGF_2\alpha$ in the ovarian artery was 30 times higher than in the iliac artery, indicating a direct transfer of $PGF_2\alpha$ from vein to artery, probably by way of a counter-current mechanism (McCracken, 1971; McCracken, Baird & Goding, 1971; McCracken, Carlson, Glew, Goding, Baird, Gréen & Samuelsson, 1972; McCracken, Baird,

Carlson, Goding & Barcikowski, 1973). Moreover, Land, Baird & Scaramuzzi (1976) compared the concentrations of $PGF_2\alpha$ in ovarian arterial blood to that in aortic and carotid blood at different stages of the oestrous cycle following infusion of radio-active $PGF_2\alpha$ into the uterine vein. They found that concentration of $PGF_2\alpha$ increased during passage through the ovarian artery proportionally to the logarithm of the concentration in the uterine vein. No such changes were observed in the concentrations of progesterone in the ovarian artery or $PGF_2\alpha$ in the uterine artery. They concluded that $PGF_2\alpha$ transferred normally from the uterine vein to the artery in sheep. Fogwell, Lewis, Butcher & Inskeep (1977) examined the transfer of $PGF_2\alpha$ from an injected follicle to a CL in the same ovary. They found that $PGF_2\alpha$ injected into a large follicle in an ovary bearing a CL is luteolytic through a local extraovarian pathway. In another experiment they observed a possible local effect of corpora lutea on follicular development within the ovary in the ewe. This finding also explains the observation that the number of large follicles and the total follicular fluid weight were greater in the ovary bearing a CL than in a non-CL bearing ovary in ewes that had ovulated unilaterally (Dufour, Ginther & Casida, 1971b, 1972). McCracken and co-workers (McCracken, 1971; McCracken, Baird & Goding, 1971; McCracken et al, 1972; McCracken, Barcikowski, Carlson, Gréen & Samuelsson, 1973) used a cross circulation preparation to investigate the problem. They studied the role of the $PGF_2\alpha$ in causing the regression of CL in ewes with either one ovary or an ovary and uterus together autotransplanted to the neck. They found that luteolytic factor in uterine venous blood at the time of luteal regress-

ion was exactly mimicked by infusion of $PGF_2\alpha$. The transport of the $PGF_2\alpha$ was also shown to take place via a vascular pathway. By introducing the levels of $PGF_2\alpha$ measured back into the uterine vein, they were able to induce premature luteal regression. Thus confirming not only the local transport of $PGF_2\alpha$ from the uterus to the ovary but also the role of $PGF_2\alpha$ as a luteolytic hormone in sheep. Further support for these findings was provided by studies in which ovine uterine vein plasma of donor sheep at different stages of the oestrous cycle was infused into the arterial supply of the ovary. Ovarian progesterone secretion was depressed only with the uterine vein plasma of day 15 (Caldwell & Moor, 1971; Baird, McCracken & Goding, 1973; Goldberg & Ramwell, 1975).

From all the foregoing experiments we can conclude that the uterus releases a local luteolytic hormone (namely $PGF_2\alpha$) which is transported locally from the uterine vein to ovarian artery apparently by a counter-current mechanism and causes the regression of the CL at the end of the oestrous cycle. However, a local extra-ovarian pathway also possibly exists between the follicle and the corpora lutea within the same ovary in sheep.

Some experimental work has failed to support the physiological role of $PGF_2\alpha$ as the uterine luteolytic factor. Thus Coudert and co-workers (Coudert, Phillips, Palmer & Faiman, 1972) failed to find consistent rises in PGF towards the end of the cycle and then later (Coudert, Phillips, Fiaman, Chernecki, & Palmer, 1974a, b) were unable to show transfer of radioactive xenon or tritium labelled $PGF_2\alpha$

from the uterine vein to the ovarian artery. Their first paper (Coudert et al.1972) was challenged and their experimental technique severely criticized by Goding (1973, 1974). Indeed it appeared from their laterwork (Coudert et al. 1974b) that some transfer of the tritium labelled $PGF_{2\alpha}$ did in fact occur, but the amount was very small. Further work is possibly needed to conclusively prove the existence of the counter-current mechanism in sheep, especially in the light of the findings of Lamond & Drost (1973). They found that sectioned of the ovarian artery distal to the region where the counter-current transfer of $PGF_{2\alpha}$ is believed to take place did not interrupt the oestrous cycle.

PROSTAGLADIN SYNTHESIS IN SHEEP

The pathways by which prostaglandins are synthesized in the uterus of sheep are still not clear but as previously discussed both oestradiol and progesterone may be necessary (see p. 66). Caldwell, Tillson, Brock & Speroff (1972) measured PGF concentrations in the peripheral plasma in ovariectomized ewes after treatment with exogenous progesterone and oestradiol. They found that PGF did not show such a significant elevation as that noticed on day 14 of the normal oestrous cycle, unless oestradiol was injected after a period of progesterone priming. The peripheral level of PGF in ovariectomized ewe rose slightly after 10 days of progesterone, indicating that progesterone alone is a weak stimulator of prostaglandin synthesis. Furthermore, low to undetect-

able levels of PGF were found in hysterectomized animals and in animals immunized against oestradiol. The oestradiol-induced increase in uterine synthesis and release of $PGF_2\alpha$ was also investigated by French & Casida (1973). They showed that intrauterine administration of 500 µg of actinomycin D, a substance which has been shown to inhibit DNA - dependent RNA synthesis (Mueller, Herranen & Jervell, 1958; De Angelo & Gorski, 1970), to sheep on day 11 of the oestrous cycle prevented CL regression. Thus regression did not occur even when 750 µg of oestradiol were injected I.M. on day 11 and 12. Furthermore, unilateral treatment of ewes bearing one CL on each ovary with actinomycin D resulted in maintenance of CL adjacent to the treated horn, when the uterine horn were ligated and severed to prevent transport of actinomycin D from treated to control horn. This results indicated that actinomycin D can delay CL regression at the end of oestrous cycle and prevent the luteolytic effect of exogenous oestradiol and also demonstrated the necessity of an essential DNA stimulation of RNA synthesis for the luteolytic action of the uterus. Thus it is possible that oestradiol may act by causing an increase in the prostaglandin synthesis in the uterus. In another study (Scaramuzzi, Boyle, Wheeler, Land & Baird, 1974), samples were collected from the uterine vein of ovariectomized ewes with the uterus transplanted to the neck after treatment with progesterone and/or oestradiol. Infusion of oestradiol alone produced no changes in the secretion of $PGF_2\alpha$ but infusion of progesterone alone had some effect. However, oestradiol infusion after pretreatment with progesterone significantly stimulated the secretion

of $PGF_2\alpha$. These results confirmed that both progesterone and oestradiol are required for the release of $PGF_2\alpha$ from the uterus of the ewe. Barcikowski, Carlson, Wilson & McCracken (1974) also confirmed these results in a similar study and furthermore, they found that the response of oestradiol acting with progesterone priming is depressed by indomethacin, an inhibitor of prostaglandin synthesis. Lewis & Warren (1975) later showed that infusion of indomethacin into the uterine horn adjacent to the CL prevented the luteolysis initiated by 750 ng of oestradiol-17β. Furthermore, Hixon, Gengenbach & Hansel (1975) suggested that oestradiol may also potentiate the luteolytic effect of $PGF_2\alpha$ in sheep. Their conclusion was based on the observation that treatment with a dose of $PGF_2\alpha$ that was luteolytic in sham-irradiated ewes failed to induce luteolysis in ewes after destruction of ovarian follicles by selective X-irradiation of the ovaries. Moreover, they reported a more rapid decrease in plasma progesterone of sham and X-irradiated ewesfollowing treatment with a combination of oestradiol and $PGF_{2\alpha}$ than was observed after treatment with either $PGF_2\alpha$ or oestradiol alone. A further study in hysterectomized and hysterectomized X-irradiated ewes after treatment with oestradiol or/and $PGF_2\alpha$ suggested that the luteolytic interaction of oestradiol and $PGF_2\alpha$ is independent of the uterus (Gengenbach, Hixon & Hansel, 1977). Although the design of this experiment does not indicate the site of action of oestradiol, the logical site would be the CL. Thus, oestradiol appears to have a dual action namely by acting with $PGF_2\alpha$ to cause luteolysis and acting on the uterus to cause a release of $PGF_2\alpha$ (Caldwell et al. 1972; Ford, Pexton, Wilson, Butcher & Inskeep, 1973; Barcikowski et al. 1974; Ford, Weems, Pitts, Pexton, Butcher & Ins-

keep, 1975). However, if oestradiol treatment following progesterone priming forms the best stimulus for $PGF_2\alpha$ release from the uterus, the level of these two hormones must increase before the release of $PGF_2\alpha$ into the plasma. Cox, Thorburn, Currie & Restall (1974) measured oestradiol and $PGF_2\alpha$ in the plasma collected from ewes prepared with indwelling catheters in both utero-ovarian veins. They found that oestradiol concentrations showed a rise about day 11 to 12, just before the first peak of $PGF_2\alpha$ on day 13. It is interesting to note that this peak coincides with the end of the first wave of follicular growth in the ovine cycle as reported by Brand & de Jong (1973). The next major rise of oestradiol occurred during pro-oestrus on day 15 and 16 and coincides with a further and more sustained output of $PGF_2\alpha$ by the uterus. This was followed by a marked decrease in progesterone until the luteolysis was complete (Cox, Thorburn, Currie, Restall & Schneider, 1973; Cox, Thorburn, Currie & Restall, 1974). It is interesting to note also that when uterine venous samples have been collected at few-hourly intervals, few workers have been able to demonstrate an increased secretion of $PGF_2\alpha$ until after the decline of luteal progesterone secretion (Thorburn, Cox, Currie, Restall & Schneider, 1973; Barcikowski, Carlson, Wilson & McCracken, 1974; Baird, Land, Scaramuzzi & Wheeler, 1976). It is clear from these data that the ovary must start secreting oestradiol just before the increase in $PGF_2\alpha$ output from the uterus and therefore oestradiol in the presence of progesterone may form the physiological stimulus for the release of $PGF_2\alpha$ from the uterus. However, the lutoelytic role of $PGF_2\alpha$ would not be complete without taking

the effect of pituitry gland into consideration (see p.39). It would appear that the survival of the CL is not just simply a matter of the presence or absence of the luteolytic hormone but depends upon the two opposing forces, the luteotrophic effect of the pituitry gland (LH & prolactin) and the embryo on the one hand and the luteolytic effect of the uterus and the follicles on the other hand. Recent studies have shown that plasma LH concentration can be increased by infusion of $PGF_2\alpha$, thus suggesting that $PGF_2\alpha$ may be involved in the process of LH release from the anterior pituitry gland of the ewe (Carlson, Barcikowski & McCracken, 1973). For example indomethacin has been found to block oestradiol-induced release of LH in anoestrous sheep (Carlson, Barcikowski, Gargill & McCracken, 1974), and systemic $PGF_2\alpha$ increases plasma LH concentration in cycling sheep (Carlson et al., 1973). Since luliberin will induce LH secretion during indomethacin blockade, it is concluded that $PGF_2\alpha$ is acting again on the hypothalamus(Roberts, Carlson & McCracken, 1976). In addition oestradial-induced release of LH in sheep is associated with increased $PGF_2\alpha$ production from the brain (Roberts, Carlson & McCracken, 1976). These results suggest that $PGF_2\alpha$ produced within the brain acts on the hypothalamus and is associated with LH release in sheep (Poyser, 1977; 1978 b).

THE MECHANISM OF THE EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON LUTEAL FUNCTION

As we have seen earlier, the exogenous administration of $PGF_2\alpha$ causes decline in progesterone secretion and structural regression of the CL in the sheep. The mechanisms by which luteolysis is brought about by $PGF_2\alpha$ remains obscure. Some studies suggest that luteolysis may be the effect of constriction of the utero-ovarian vein which result in reduction of the blood flow to the CL. Pharriss, Cornette & Gutknecht (1970) initially proposed that $PGF_2\alpha$ can significantly reduce blood flow in the utero-ovarian vein and thus decrease the blood flow to the ovaries. Thorburn & Hales (1972) confirmed this suggestion in sheep by measuring the blood flow in the CL and ovarian tissue during infusion of $PGF_2\alpha$ into a uterine vein on day 8-9 of the oestrous cycle. Moreover, Intrauterine or I.M. injection of $PGF_2\alpha$ resulted in premature regression of CL (Douglas & Ginther, 1973; Nett, McClellan & Niswender, 1976) with a reduction in ovarian blood flow similar to that observed during normal regression (Mattner, Hales & Brown, 1972; Niswender, Diekman, Nett & Akbar, 1973, Bruce & Moor, 1976; Niswender, Reimers, Diekman & Nett, 1976). Baird (1974) infused $PGF_2\alpha$ into the ovary through the ovarian artery in ewes with autotransplanted ovaries. He found that the mean ovarian blood flow fell by 10-15% during the infusion, during which there was a dramatic decline in secretion of progesterone. Similarly, the data of Nett, McClellan & Niswender (1976) also indicated that administration of $PGF_2\alpha$ reduced blood flow to the CL-bearing ovary and simultaneously reduce the secretion of progesterone.

However, changes in blood flow similar to that of normal ewes were produced in anoestrous and ovariectomized ewes by treatment with progesterone and oestrogen (Brown, Hales & Mattner, 1974), suggesting a primary interaction of oestrogen and progesterone on the blood flow. Also, Einer-Jensen & McCracken (1976) reported that luteolysis induced by $PGF_2\alpha$ administration in sheep is not accompanied by the blood flow changes in the CL. Horton & Poyser (1976) suggest that the reduction in the luteal blood flow may be the result but not the cause of luteal regression and also pointed out that an effect on the vascular system would not account for the inhibition of progesterone production observed in vitro.

Studies in sheep suggest that $PGF_2\alpha$ affected both the structural and functional (biochemical) changes of the CL. Structural luteolysis was found to follow the functional luteolysis (Umo, 1975).

Many workers studied the structural changes in the CL of the sheep during luteal regression, using both light and electron microscopy (Warbritton, 1934; Deane, Hay, Moor, Rowson & Short, 1966; Bjersing, Hay, Moor, Short & Deane, 1970; Thwaites & Edey, 1970; Gemmell, Stacy & Thorburn, 1974a,b, 1976; Stacy, Gemmell & Thorburn, 1975; 1976; Umo, 1975; Stacy & Gemmell, 1976a, b). All these studies concentrated on changes in the luteal cell. However, a few other studies are available which deal with changes in the blood vessels. Bjersing et al. (1970) reported the presence of cellular debris within the capillaries of regressed CL. Similar debris was demonstrated more recently be Chamley & O'Shea (1975) in corpora lutea

which were regressing following the injection of $PGF_2\alpha$ into them. Furthermore, Niswender et al. (1976) has shown a decrease in relative volume of the capillary network within the CL during regression. O'Shea, Nightingale & Chamley (1977) studied the changes in the small blood vessels of the corpora lutea during cyclical regression by both light and electron microscopy. They concluded that at a relatively early stage of luteolysis, many luteal capillaries show changes involving obstruction of their luminae by large amounts of cellular debris at least part of which is of endothelial origin. Also McClellan, Abel & Niswender (1977) have shown a number of morphological and biochemical events associated with luteolysis are similar during normal and exogenous $PGF_2\alpha$ induced regression.

Other studies have suggested a direct effect of $PGF_2\alpha$ on the CL. Henderson & McNatty (1975) found that $PGF_2\alpha$ inhibited steroidogenesis and initiated regression of CL by inhibiting the adenyl cyclase sytem. However, PGE_2 enhanced the functions of adenyl cyclase sytem and overcame the effect of $PGF_2\alpha$ (Henderson & McNatty, 1975; Henderson, Scaramuzzi & Baird, 1977), which may account for the antagonistic effect of PGE_2 when injected along with $PGF_2\alpha$ (Henderson et al. 1977; Mapletoft et al. 1977).

The newly formed CL of sheep was suggested to be refractory to the lytic action of $PGF_2\alpha$ (Hearnshaw, Restall & Glesson, 1973). This phenomenon was studied in vitro by Henderson & McNatty (1977). Since the steroidogenic potential of the granulosa-luteal cell is related to the amount of LH bound to the cell, the bound LH may

protect the cells from the lytic action of $PGF_2\alpha$. Thus, Henderson & McNatty (1977) postulated an interaction between LH and $PGF_2\alpha$ to account of the resistance of the newly formed CL to $PGF_2\alpha$. Furthermore, Niswender et al. (1976) show that treatment of ewes with anti-LH serum resulted in a rapid decline in blood flow to the ovary as well as in circulating progesterone concentrations. While infusion of exogenous LH resulted in an increase in serum concentration of progesterone associated with a small increase in blood flow to the ovary containing the CL.

The data of Diekman, O'Callaghan, Nett & Niswender (1978a) demonstrated that administration of $PGF_2\alpha$ to the sheep on day 10 of the cycle signficantly decreased the total number of LH receptors within 22.5 hr. The number of receptors occupied by endogenous LH was also reduced at 22.5 hr after $PGF_2\alpha$ administration. All parameters of the functional corpora lutea measured 22.5 hr after $PGF_2\alpha$ treatment are comparable to those observed at the last day of the oestrous cycle (Dickman, O'Callaghan, Nett & Niswender, 1978b). It has been hypothesized that the biochemical event that initiates luteolysis is the decline in the concentration of luteal receptors. Nett, McClellan & Niswender (1976) have shown that administration of $PGF_2\alpha$ to ewe at the mid-luteal phase of the oestrous cycle significantly decreases progesterone concentrations within 6 hr. However, this decline occurred before the decrease either in unoccupied or occupied LH receptors (Diekman et al. 1978a). Although decreased numbers of both total and occupied LH receptors are associated with $PGF_2\alpha$-induced luteolysis, the timing of these decreases is not compatible with this being the mechanism whereby $PGF_2\alpha$ induces luteal regression.

Other changes in the CL are more closely associated with diminished progesterone secretion and luteolysis (Nett, McClellan & Niswender, 1976). Furthermore, the observation that $PGF_{2\alpha}$ blocked LH stimulation of progesterone production and cyclic AMP production by rat luteal tissue (Lahav, Freud & Lindner, 1976) led other workers (Behrman, Grinwich, Hichens & Macdonald, 1978) to study the time course of the effect of $PGF_{2\alpha}$ in vivo on radio-labelled gonado trophin accumulation by luteal tissue. They reported that the rapid drop in plasma progesterone levels produced by $PGF_{2\alpha}$ was associated with a decrease in labelled gonadotrophin uptake by corpora lutea in the first two hours after $PGF_{2\alpha}$ administration but not from loss of gonadotrophin receptors. However, there was a significant reduction in the number of LH receptors within 24 hours after $PGF_{2\alpha}$ administration as has also been observed in sheep.

From all this data we conclude that the luteal regression is caused by a biochemical action of $PGF_{2\alpha}$ on the luteal cells, possibly assisted by an effect on blood flow. Whether the luteolytic activity of $PGF_{2\alpha}$ is connected with its ability to bind with $PGF_{2\alpha}$ receptors in the ovary is not known, though receptors for $PGF_{2\alpha}$ are present in the corpus luteum of the sheep, cow, mare and human (Powell, Hammerström & Samuelsson, 1974; Powell, Hammarström, Samuelsson & Sjöberg, 1974; Rao, 1974, Kimball & Wyngarden, 1977). However, very recently, Rao, Estergreen, Carman & Moss (1979) found that it is the increase in the affinity of rather than the number of receptors which control the effect of $PGF_{2\alpha}$ at the time of luteal regression. Moreover they reported that luteal $PGF_{2\alpha}$ binding was low in the early luteal phase, progressively increased to reach a high value at the time when functional luteolysis was actively occurring and then declined when both structural and functional luteolysis was complete.

Role of oxytocin in luteolysis

Oxytocin treatment of cows during the first third of the oestrous cycle has been found to shorten the cycle length and this shortening effect is abolished by hysterectomy (Armstrong & Hansel, 1959; Hansel & Wagner, 1960). Luteolysis induced by oxytocin only occurred in the hemihysterectomized animal when the ovary containing the CL was ipsilateral to the uterine horn left in situ (Ginther, Woody, Mahajan, Jarakiraman & Casida, 1967). These data along with the finding in the rat, that oxytocin treatment has been shown to cause the release of prostaglandin-like substances from the isolated uterus in vitro (Chan, 1974) suggest a role for oxytocin in luteolysis.

However, the role of oxytocin in luteolysis in the sheep is somewhat confused. There are no reports that oxytocin injections can shorten the oestrous cycle in the sheep as it does in the cow, but both Sharma & Fitzpatrick (1974) and Roberts and co-workers (Roberts, Barcikowski, Wilson, Skarnes & McCracken, 1975; Roberts & McCracken, 1976) reported an increase in $PGF_2\alpha$ release from the uterus following oxytocin administratio Roberts and co-workers (Roberts et al., 1975; Roberts & McCracken, 1976) found that infusion of oxytocin into the uterine artery of the sheep in the latter part of the cycle significantly increased the rate of $PGF_2\alpha$ secretion. They assumed this to be a direct effect of oxytocin on the uterus as in vitro studies showed that oxytocin increased $PGF_2\alpha$ release by the ovine endometrial tissue (Roberts, McCracken, Gavagan & Soloff, 1976). However, Sharma & Fitzpatrick (1974) could not demonstrate an effect of oxytocin infusion alone, but found that oxytocin potentiated the release of $PGF_2\alpha$ by oestrogen. The reason for this discrepancy is unclear. Roberts and co-workers (Roberts et al., 1975; Roberts & McCracken, 1976) further showed that oxytocin-induced release of $PGF_2\alpha$ in vivo was not due to a side effect of uterine contractions as indomethacin inhibited the release of $PGF_2\alpha$ but not uterine contractions, even though administration of $PGF_2\alpha$

alone mimic the effect of oxytocin on uterine smooth muscle. Furthermore, Mitchell & Fint (M.D. Mitchell & A.P.F. Fint in Poyser & Maule Walker, 1979) found that immunization of sheep against oxytocin extended the length of oestrous cycle. Moreover, Roberts, McCracken, Gavagan & Soloff (1976) found that the synthesis of $PGF_2\alpha$ by the ovine uterus may involve interaction between oxytocin and its endometrial receptors and suggest that oxytocin could play a role in luteal regression by contributing to the regulation of the uterine synthesis of $PGF_2\alpha$. Oestrogen may also influence prostaglandin synthesis by regulating the availability of the endometrial oxytocin-receptors in sheep (Roberts, McCracken, Gavagan & Soloff, 1976). Soloff (1976) has provided direct evidence in ovariectomized rats, that oestrogenic substances lead to enhanced binding of oxytocin by the uterus. Thus oestrogen may influence the secretion of prostaglandins from the uterus not only by conditioning prostaglandin-synthetic mechanisms but also by inducing the development of oxytocin-receptors which, when activated by oxytocin, increased the amounts of $PGF_2\alpha$ produced by the endometrium. This concept would explain the finding reported by Sharma & Fitzpatrick (1974), that oxytocin failed to stimulate the uterus of the anoestrous ewe to release $PGF_2\alpha$ unless the animal was first primed with oestradiol-17β.

These data suggest that oxytocin has a luteolytic action by causing the release of $PGF_2\alpha$ from the uterus and it is possible that $PGF_2\alpha$ released during oxytocin action may have other important physiological functions (i.e. by altering blood flow). Further study is needed to verify the role of oestrogen in the response of the $PGF_2\alpha$ synthetic mechanisms to oxytocin and so clarify the role of oxytocin in luteolysis during oestrous cycle.

EXPERIMENTAL STUDIES

The experiments reported in the thesis were initiated in 1976. The information available at that time left some doubt on the part of the pathway of prostaglandin synthesis at which extra-uterine factors acted to cause the increase in prostaglandin $F_2\alpha$ release needed for luteolysis in sheep , although some progress had been made towards identifying the factors responsible for the release of luteolytic hormone, $PGF_2\alpha$, at the end of oestrous cycle (Horton & Poyser, 1976).

Studies involving measurement of prostaglandins and steroids hormones as well as the injection of these hormones during the latter part of the oestrous cycle in sheep suggested that the uterus plays a role in controlling luteal function after day 12 of the oestrous cycle. Events occur at this time that cause the CL to start to regress. Thus it appears that some change occurs during this period of the cycle to cause the production of the luteolytic hormone. Oestradiol and/or progesterone appear to play an important role in the mechanism of production of prostaglandins in uterine tissue and their release into the blood as well as into the uterine fluid.

The purpose of the present experiments was to:

1. Investigate the distribution of prostaglandin $F_2\alpha$, PGE_2 and 6-oxo-$PGF_1\alpha$ in uterine tissue.

2. Make regular and simultaneous measurements of uterine

content of $PGF_2\alpha$ and PGE_2, the ability of endometrial tissue to synthesis these prostaglandins and the concentration of $PGF_2\alpha$ in uterine venous blood of the utrine horn to find the relationships between these parameters.

3. Measure $PGF_2\alpha$ and progesterone in the different blood vessels in the tubal area of the uterus to consider the pathways operating.

Incidental to the collection of uterine fluid for evaluating the 6-oxo-$PGF_1\alpha$ assay a small investigation into the effect of progesterone injection on uterine prostaglandin production and release was undertaken.

Previous work using non-pregnant sheep with the uterine vein cannulated to the exterior had shown that this method was unreliable for long term sampling of uterine venous blood (see p. 29). It was felt that the method reported by Thorburn & Mattner (1969, 1971) of diverting the uterine venous drainage through an accessible vein such as the anterior mammary vein would prove more reliable. This was achieved by performing an end-to-end anastomosis of the utero-ovarain vein to the anterior mammary vein. Thus the uterine venous blood was diverted through the ventral abdominal wall along the anterior mammary vein situated superficially in the midline. However, the unusual properties of the anterior mammary vein have to be taken into account. In most virgin female sheep the valves in the anterior mammary vein point towards the udder, thus allowing only a caudal flow of blood (Linzell, 1960). During lactation some or all

of the valves become incompetent and the blood flow in mammary vein tends to flow cephalicly. Thus in the present experiments we tried to avoid using virgin females and used only ewes which had lambed at least once. In such animals the direction of blood flow in their mammary vein was always away from the udder.

The collection of uterine biopsy samples proved to be possible by fistulating one uterine horn to the exterior. Such surgery was necessary as access to the uterus in anaesthetized ewes as well as conscious animals through the external opening of the reproductive system proved to be difficult. An instrument designed for such a purpose would have to pass through all different parts of the reproductive system, i.e. vulva (2.5 - 3 cm in length), vagina (8 cm), cervix (4 cm), to the body of the uterus. However, the main obstacle is the complicated structure of the cervix which may consist of a series of some 5-8 interlocking valves (see Fig 5). As regular endometrial biopsies via the os cervix were not possible a more drastic method involving fistulation of one uterine horn to the exterior was adopted. Surprisingly, infection did not prove to be a problem and the removal of endometrial biopsies was straightforward.

Figure 5

A photograph showing the reproductive tract of the ewe opened longitudinally. 1 - the base of the uterine horn, 2 - the cervix and its interlocking valves, 3 - the upper part of the vagina.


1
2
3
0
1
2
3cm

EXPERIMENT 1: Anastomosis of uterine vein to the anterior mammary vein.

Objective:

This experiment was undertaken to sample the uterine venous blood, now draining into the superficial anastomosed utero-mammary vein, to confirm the episodic release of $PGF_2\alpha$ found by previous workers.

Preparation of Animals and Anaesthesia

A single mature non-pregnant Dorset Horn x Finnish Landrace ewe (ewe 76) was used. Oestrus was detected by daily testing with a vasectomized ram. The first day of oestrus was taken as day 1 of the cycle. The ewe was kept in an individual pen under natural daylight and fed about 1500 g. of hay daily.

Food was withheld for 24 hr before surgery. Anaesthesia was induced with nitrous oxide and halothane and maintained on halothane, nitrous oxide and oxygen mixture. The operation was carried out with full aseptic surgical procedures.

Vessel Anastomosis

The experimental procedure involved end-to-end anastomosis of the left uterine vein to the anterior mammary vein (vena epigastrica cranialissuperficialis) by a method adapted from that of Thorburn & Mattner, (1971). However, in the current experiment

the utero-ovarian vein and artery were not separated, but instead the adjacent ovary was removed.

A skin incision was made about 2 cm lateral to the anterior mammary vein. The skin flap was lifted and the anterior mammary vein carefully dissected free of subcutaneous tissue. Once freed, the vein was placed back under the skin flap to reduce venous spasm. The abdomen was opened through a paramedian incision directly beneath the skin incision. The genital tract was located and inspected and the left ovary removed. The left uterine vein and associated ovarian artery were separated from the mesometrium, ligated as close to the posterior vena cava as possible and severed. The anterior mammary vein was then ligated close to the udder and also severed. The small artery which often runs alongside the anterior mammary vein was separated and tied off. A small elliptical hole was cut in the linea alba a few centimetres to the left of midline and the severed end of the mammary vein passed through it. The anterior mammary vein was then anastomosed to the utero-ovarian vein using Nakayama's instrument for small vessel anastomosis (Senko Medical Instrument, Mfg. Co. Ltd., Bunkyoku, Tokyo, Japan) (see Fig 6A,B).

After ensuring complete haemostasis, the abdominal and skin incisions were closed with mattress sutures of catgut (chromin 116; Ethicon Ltd., Edinburgh) and nylon (Ethicon 186; Ethicon Ltd., Edinburgh) respectively. This procedure resulted in the uterine venous drainage being diverted as shown in Fig 7.

Figure 6

A photograph showing the Nakayama's instrument for small vessel anastomosis (Senko Medical Instruments, Mfg. Co. Ltd, Bukyoku, Tokyo, Japan).

A - anastomosis equipment consisting primarily of two rings holder (top 2 instruments) and a pair of pliers.
B - the rings used to join the vessels are shown enlarged.

The full instructions for these instruments are included in Appendix 11, for information.

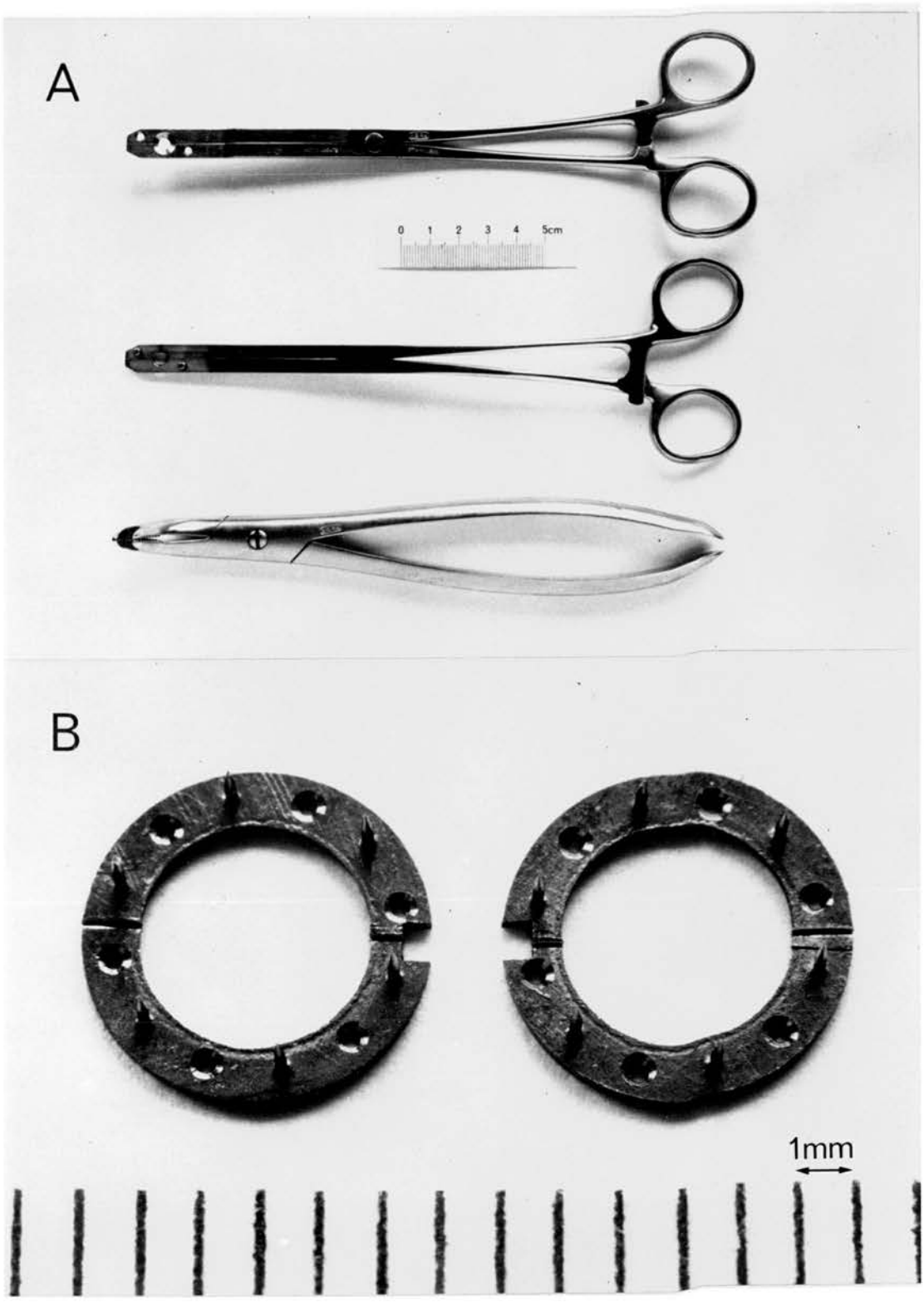
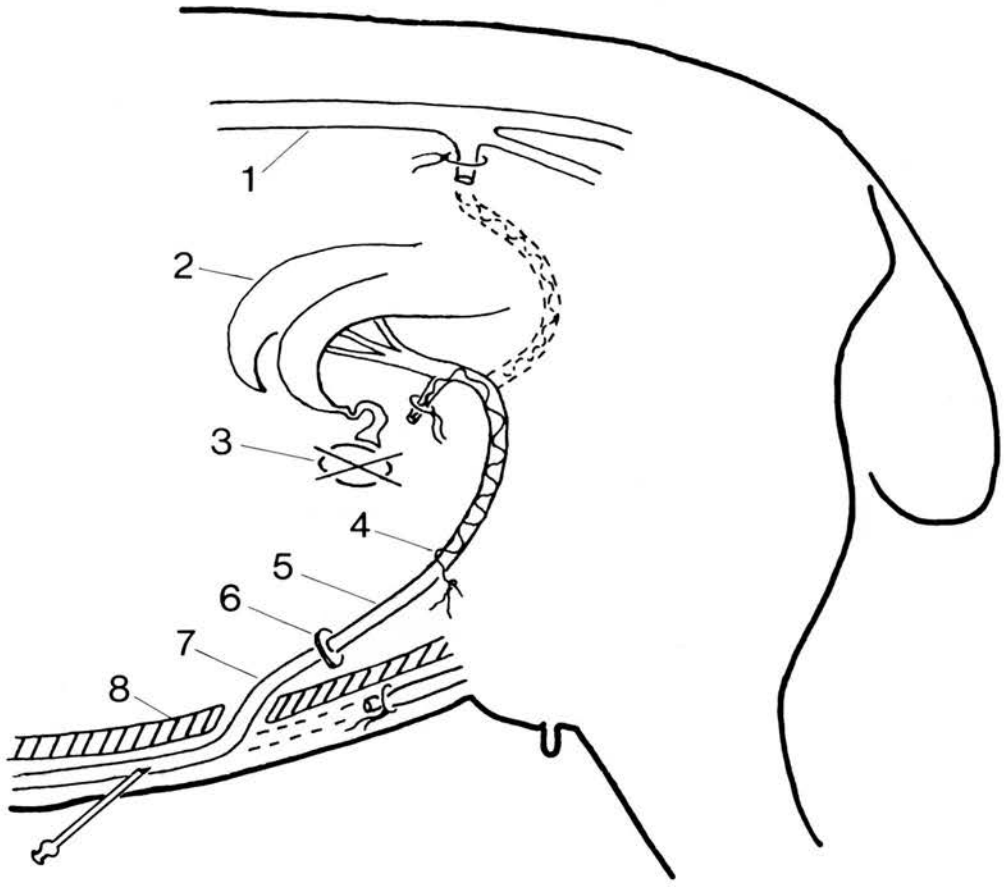

A
0 1 2 3 4 5cm
B
1mm

Figure 7

Diagram of the anastomosis between the left uterine vein and the anterior mammary vein in the sheep. 1 - posterior vena cava, 2 - uterus, 3 - ovary, which is removed, 4 - ovarian artery, 5 - utero-ovarian vein, 6 - anastomosis rings, 7 - anterior mammary vein, 8 - abdominal wall.


1
2
3
4
5
6
7
8

When healing was complete, the patency of anastomosis was checked by retrograde injection of 5 ml of Urografin 76% (Schering Chemicals Ltd., Burgess Hill, Sussex) into the anastomosed utero-mammmary vein and the vessels visualised by X-radiography (Fig 8A). This checking was infact done some 5 months after surgery due to the intervention of the summer "anoestrus".

As the anastomosis was patent, samples of uterine venous blood could thus be obtained by simple venipuncture from the anastomosed utero-mammary vein. At post-mortem the patency of the anastomosis was rechecked by injection of coloured gelatin solution (Fig 8B,C).

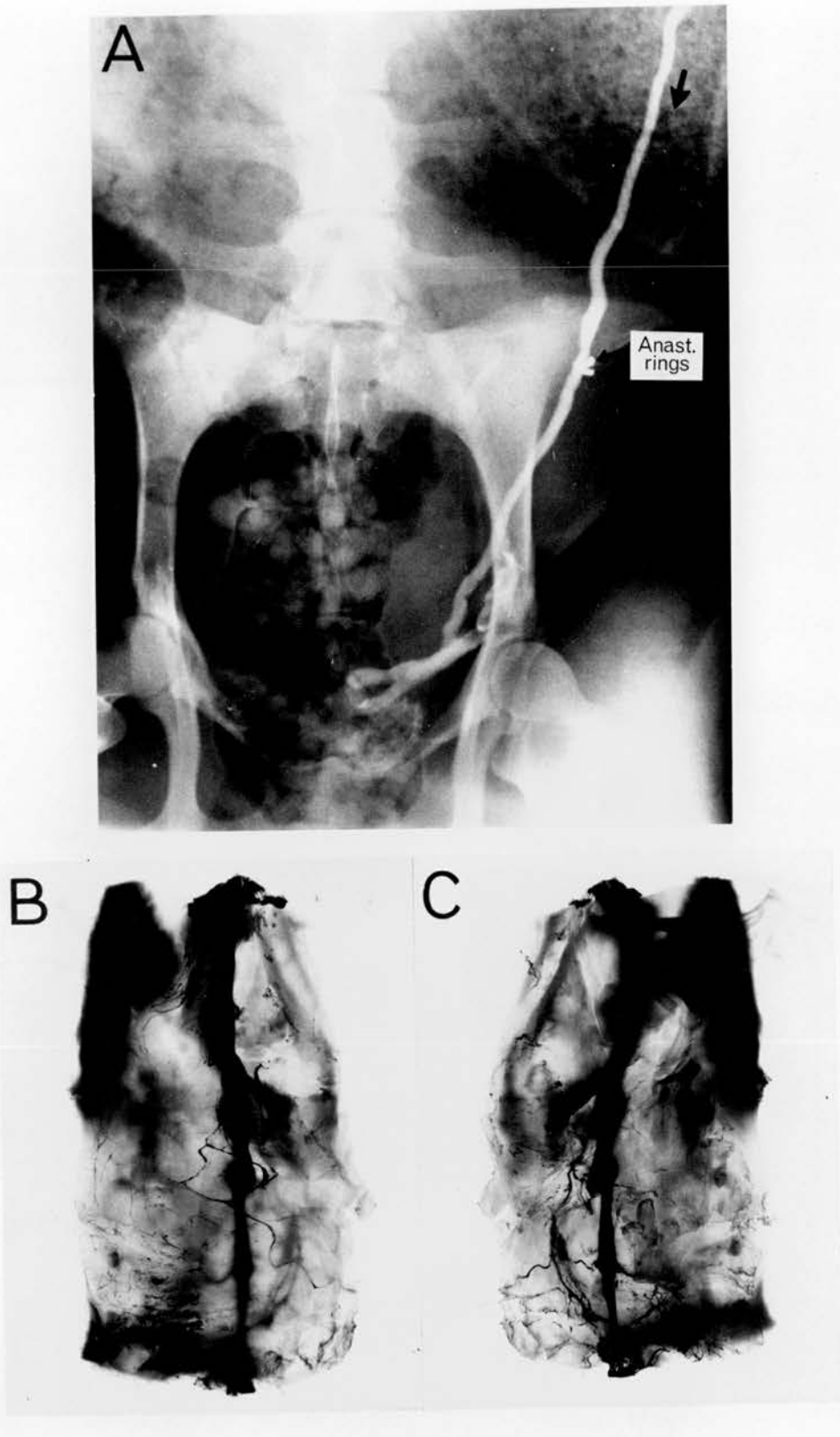
Samples

Daily plasma samples were taken at approximately 10.00 hr from day 3 to 11 of the oestrous cycle. Then more frequent samples (every 3 hours) were taken from day 12 to the onset of the next oestrus. At each sampling 3 ml of uterine venous blood were taken by venipuncture from the anastomosed utero-mammary vein. Blood samples were centrifuged at 1500 g (3500 rpm) for 10 mins, and the plasma was removed and stored at -20°C. Progesterone and $PGF_2\alpha$ concentrationsin plasma were determined by radioimmunoassay at the Department of Pharmacology, University of Edinburgh, using methods previously described (Blatchley & Poyser, 1974; Dighe, Emslie, Henderson, Rutherford & Simon, 1975; Poyser & Horton, 1975; Fenwick, Jones,Naylor, Poyser & Wilson, 1977). The one-step extraction procedures that were used gave greater than 90% recovery of $PGF_2\alpha$ and

Figure 8

A photograph showing the patency of the utero-mammary vein anastomosis of ewe 76.

A - radiograph showing injection of 5 ml of Urografin 76% (Schering Chemicals Ltd., Burgess Hill, Sussex). B & C - photograph showing dorsal (B) and ventral (C) views of Toluene cleared preparation of the abdominal muscle of ewe 76 following injection of coloured gelatin solution at post-mortem. The midpoint of the pictures shows the patent vessel passing through the abdominal muscle layer.


A
Anast.
rings
B
C

progesterone. Within assay coefficients of variation were 12.3 and 12.1% for progesterone and $PGF_2\alpha$ respectively. The between assay coefficients of variation were 11.2 and 10.3% respectively The limits of detection of the assays were, progesterone, 20 pg; $PGF_2\alpha$, 40 pg.

RESULTS

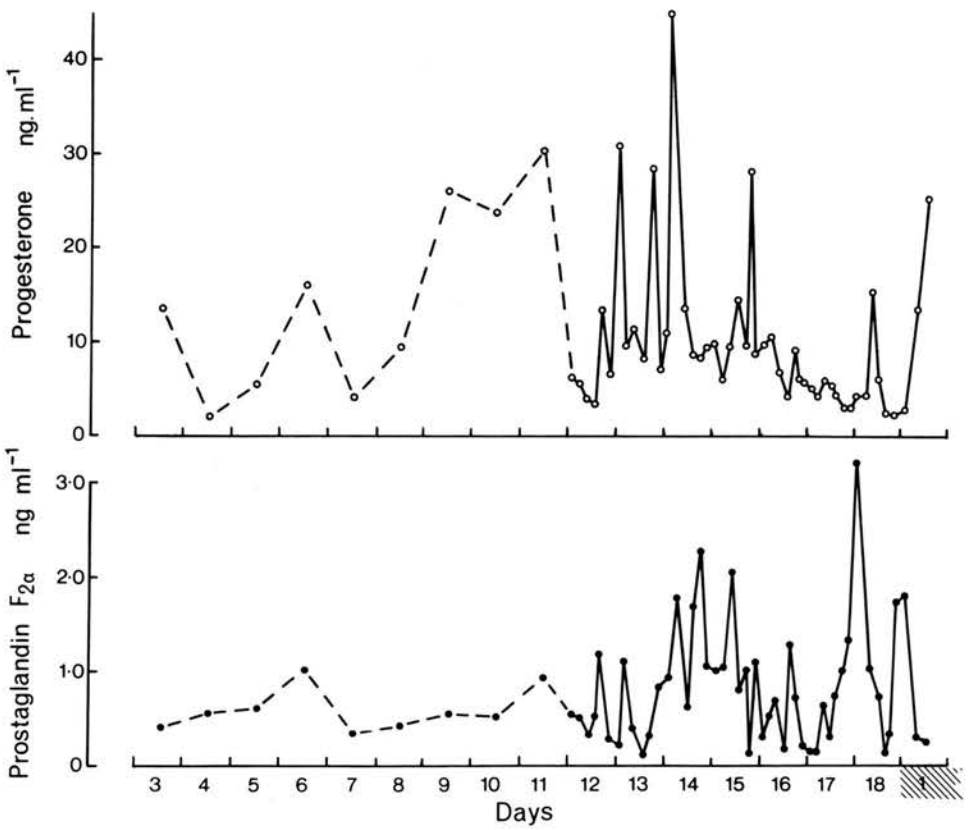
The changes in the concentration of $PGF_2\alpha$ and progesterone in uterine venous blood collected from the patent utero-mammary vein during the oestrous cycle are shown in Fig 9. The ewe returned to oestrus after a normal 18-day cycle. Daily sampling gave the impression of relatively smooth changes in plasma levels of $PGF_2\alpha$ and progesterone. However, more frequent samples showed that the concentrations of both hormones fluctuated episodicly. $PGF_2\alpha$ showed a complex series of peaks from day 14 to 18 of the cycle. The first large peak of $PGF_2\alpha$ occurred on day 14 and others followed through day 14 and 15. These were followed by a steady increase in $PGF_2\alpha$ concentration on day 17 to reach a maximum (as high as 3.24 ng ml^{-1}) on day 18 at a time when luteolysis was almost complete. The mean plasma progesterone concentration declined steadily from day 16 to 18 indicating the course of luteal regression in the ovary on the opposite side.

DISCUSSION

The anastomosis method between the uterine vein and the anterior mammary vein used in the present experiment was adapted

Figure 9

Diagram of simultaneous concentration (ng ml^{-}) of progesterone (open circles) and prostaglandin $F_{2\alpha}$ (solid circles) in uterine venous blood collected from a patent anastomosed utero-mammary vein during the oestrous cycle in ewe 76. Daily samples were taken from day 3 to 11 and more frequent (every 3 hr) samples were taken from day 12 to onset of oestrus. The animal had a normal 18-day oestrous cycle.


Progesterone ng.ml⁻¹
40
30
20
10
0
Prostaglandin F2α ng ml⁻¹
3·0
2·0
1·0
0
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Days

from that of Thorburn & Mattner (1971). Since the luteolytic substance was suggested to pass from the uterine vein to the ovarian artery by a counter-current mechanism and thus caused a regression of CL only in adjacent ovary (see p.78), the separation of these two vessels would prevent the luteolytic substance from reaching CL and would result in their maintenance. In view of this, the removal of the adjacent ovary was considered necessary to ensure normal oestrous cycles in the current experiments.

The fluctuations in progesterone concentrations are difficult to interprete in view of the absence of the ovary. However, the decline in mean progesterone levels from day 16 onwards indicated a normal luteal regression in the remaining ovary. The episodic pattern of $PGF_2\alpha$ release agrees very closely with that found in the uterine venous blood collected from ewes either with the uterus left in situ (Thorburn, Cox, Currie, Restall & Schneider, 1972; 1973; Cox, Thorburn, Currie, Restall & Schneider, 1973) or with utero-ovarian autotransplant (Baird, Land, Scaramuzzi & Wheeler, 1976). As both Thorburn & co-workers and Baird & co-workers employed sheep with indwelling catheters into utero-ovarian vein, the close similarity between their results and those found here confirm the validity of the anastomosis method for measuring the concentration of different hormones in the uterine venous blood.

EXPERIMENT II: Distribution of prostaglandins ($PGF_2\alpha$, PGE_2 and 6-oxo-$PGF_1\alpha$) in uterine tissue.

Objective:

The purpose of this experiment was to take simultaneous tissue samples from the different layers of the uterus i.e. caruncular, non-caruncular endometrium and myometrium throughout the oestrous cycle. The measurement of the content of $PGF_2\alpha$ and PGE_2 in this tissue and the ability of this tissue to synthesize these prostaglandins should throw some light on the part of the uterus responsible for secretion of $PGF_2\alpha$ and PGE_2 during the oestrous cycle. In view of the work reported by Fenwick, Jones, Naylor, Poyser & Wilson (1977) and Jones, Poyser & Wilson (1977) who found 6-oxo-prostaglandin $F_1\alpha$ (6-oxo-$PGF_1\alpha$) in the uterine tissue of rat, guinea-pig and sheep , the concentration of 6-oxo-$PGF_1\alpha$ was also measured in the different uterine tissue on various days of oestrous cycle to see if it played any role in the luteolytic process.

Animals and Samples

Samples of uterine tissue were taken from 10 mature non-pregnant Dorset Horn x Finnish Landrace ewes (ewe 64, 76, 86, 87, 88, 89, 93, 94, 105 & 111) by laparotomy under anaesthesia on various days of the oestrous cycle. Anaesthesia was induced with either intravenous injection of 20 ml Saffan (alphaxalone & alphadolone acetate, Glaxo Laboartories Ltd., Greenford, Middlesex) or

with nitrous oxide and halothane. The animals was maintained on halothane, nitrous oxide and oxygen. In all cases duplicate samples (each greater than 20 mg) of caruncular, non-caruncular endometrium and myometrium were taken.

Methodsof Extraction

To ascertain the initial prostaglandin content of the endometrial tissue, one sample of each tissue was immediately placed in 5 ml absolute ethyl alcohol and stored at -20°C. This sample was later homogenized in the alcohol in which it had been stored and centrifuged. The supernatant was withdrawn and the precipitate washed in 5 ml absolute ethanol, recentrifuged and the washing were combined with the original extract. The alcoholic extract was evaporated to dryness and the residue was dissolved in 10 ml water. The pH of the aqueous extract was then lowered to 4.5 and the prostaglandins were extracted as described below and stored in 2 ml ethyl acetate at -20°C until assayed. To obtain an indication of the ability of the uterine tissue to synthesize $PGF_{2\alpha}$ and PGE_2, albeit under in vitro conditions, the other tissue sample was homogenized in 15 ml Tyrode's solution with a Fison's glass homogenizer and was incubated for 90 minutes at 37°C with a constant stream of oxygen bubbling through it. Prostaglandins synthesis was halted after incubation by adjusting the pH to 4.5 with M HCl. The sample was extracted three times with 40 ml redistilled ethyl acetate and the three fractions were combined. After washing with distilled water (pH lessthan 6.0) the combined

fraction was evaporated to dryness and the residue redissolved in 5 ml ethyl acetate and stored at -20°C until analysed. $PGF_2\alpha$, PGE_2 and 6-oxo-$PGF_1\alpha$ in extracts of uterine tissue were measured by radioimmunoassay using the methods based on those described previously (Blatchley & Poyser, 1974; Dighe, Emslie, Henderson, Rutherford & Simon, 1975; Poyser & Horton, 1975:Fenwick, Jones, Naylor, Poyser & Wilson, 1977). Again the one-step extraction procedures that were used give greater than 90% recovery of $PGF_2\alpha$ and PGE_2 and 65% of 6-oxo-$PGF_1\alpha$. Within-assay coefficients of variations were 12.1 and 11.9% for $PGF_2\alpha$ and PGE_2 respectively, and the between-assay coefficeints of variations were 10.3 and 13.5% respectively. The within- and between-assay coefficients of variations for 6-oxo-$PGF_1\alpha$ were not detected due to insufficient samples. The limits of detection of the assays were, $PGF_2\alpha$, 40 pg; PGE_2, 4 pg and 6-oxo-$PGF_1\alpha$, 40 pg.

RESULTS

The results of this experiment are summarized in Fig 10 and given in more detail in Tables I and II. As can be seen from Table I, endometrial $PGF_2\alpha$ content in both caruncular and non-caruncular tissue increase signficantly towards the end of oestrous cycle (Caruncular tissue, Day 3-8 $\underline{V}$ day 12-16, $p<0.02$; Non-caruncular tissue, day 3-8, $\underline{V}$ day 12-16, $p<0.01$). PGE_2 content only increased significantly in the caruncular part of the endometrial tissue (Day 3-8 $\underline{V}$ day 12-16, $p<0.05$).

Figure 10

Diagram of the means and standard error of the means (S.E.M.) of content (ng mg^{-1}) and synthesizing ability (ng mg^{-1} 90 min^{-1}) of $PGF_2\alpha$ (solid bars), PGE_2 (hatched bars) and 6-oxo-$PGF_1\alpha$ (open bars) for myometrium and caruncular and non-caruncular endometrium of the uterine tissue in sheep. The figures in brackets indicate the number of animals in each group. Columns marked with one asterisk (*) are signficantly greater than those marked with two (**).

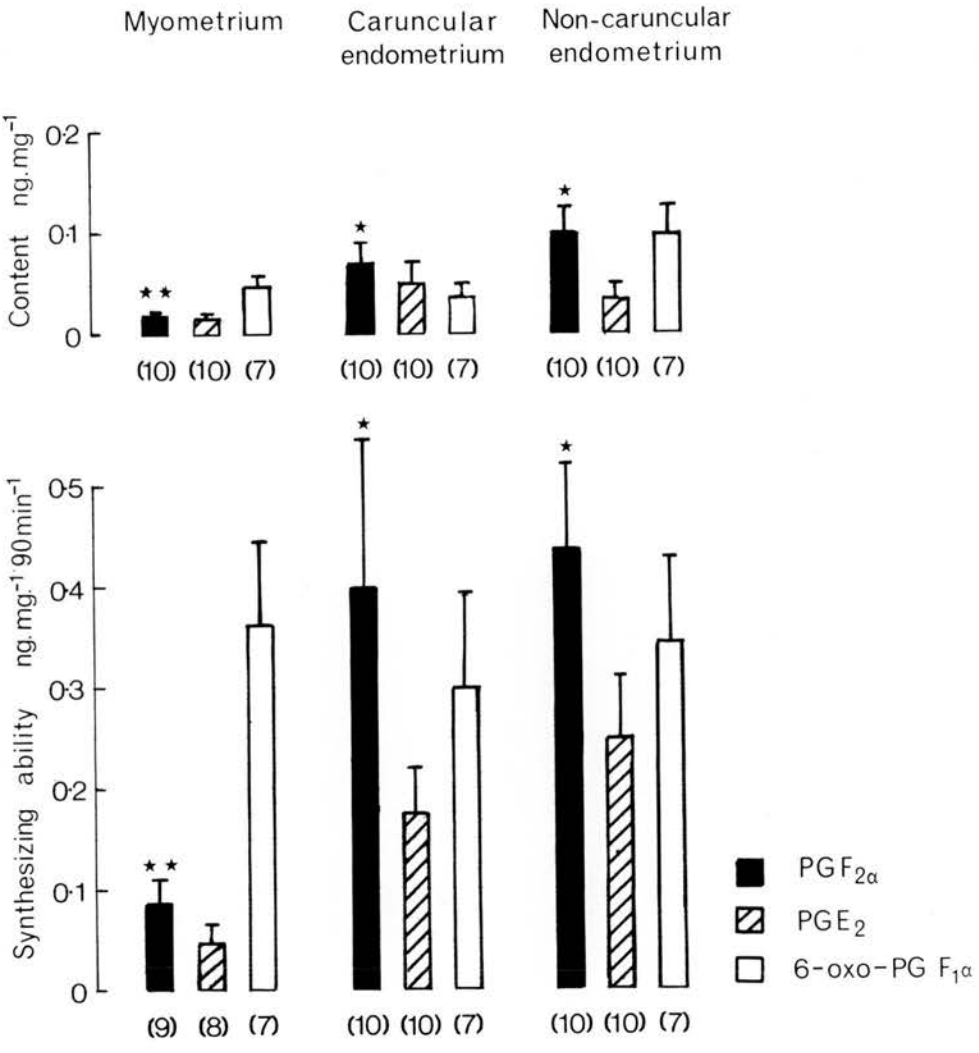

Myometrium
Caruncular endometrium
Non-caruncular endometrium
Content ng.mg-1
0·2
0·1
0
(10) (10) (7)
(10) (10) (7)
(10) (10) (7)
Synthesizing ability ng.mg-1 90min-1
0·5
0·4
0·3
0·2
0·1
0
(9) (8) (7)
(10) (10) (7)
(10) (10) (7)
PGF2α
PGE2
6-oxo-PG F1α

TABLE 1: The prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and prostaglandin E_2 (PGE_2) content and synthesizing ability of uterine myometrium and caruncular and non-caruncular endometrium in sheep.

Ewe No.	Day of Oestrous Cycle	Myometrium		Caruncular Endometrium		Non-caruncular Endometrium	
		a $PGF_2\alpha$	b PGE_2	c $PGF_2\alpha$	d PGE_2	e $PGF_2\alpha$	f PGE_2
Tissue Content (ng mg^{-1})							
76	3	0.019	0.011	0.004	0.001	0.004	0.001
89	5	0.004	0.008	0.013	0.009	0.028	0.009
93	5	0.020	0.050	0.060	0.010	0.030	0.008
86	7	0.010	0.010	0.012	0.060	0.014	0.020
105	8	0.017	0.028	0.024	0.012	0.056	0.028
64	12	0.010	0.006	0.050	0.012	0.100	0.021
111	13	0.019	0.017	0.051	0.034	0.132	0.074
87	15	0.030	0.017	0.150	0.022	0.170	0.021
88	15	0.009	0.004	0.170	0.110	0.230	0.150
94	16	0.040	0.006	0.160	0.030	0.230	0.030
Synthesizing Ability (ng mg^{-1} 90 min^{-1})							
76	3	0.05	0.02	0.10	0.02	0.18	0.13
89	5	0.01	low	0.25	0.15	0.46	0.24
93	5	0.08	0.16	0.44	0.16	0.36	0.14
86	7	0.06	0.02	0.15	0.17	0.20	0.12
105	8	0.07	0.04	0.14	0.82	0.29	0.10
64	12	-	-	0.33	0.18	0.44	0.24
111	13	0.05	0.01	0.08	0.06	0.28	0.15
87	15	0.23	0.04	1.62	0.58	1.10	0.84
88	15	0.08	0.04	0.36	0.20	0.48	0.21
94	16	0.15	0.01	0.47	0.16	0.61	0.24

The statistical significance of the differences between $PGF_2\alpha$ values in the different uterine tissues and also between PGE_2 values in the different tissues:

Tissue Content: a v c $p<0.02$; a v e $p<0.02$
b v d $p<0.3$; b v f $p<0.3$

Synthesizing ability: a v c $p<0.05$; a v e $p<0.01$
b v d $p<0.1$; b v f $p<0.1$

TABLE II: The 6-oxo-$PGF_{1\alpha}$ content and synthesizing ability ot uterine myometrium and caruncular and non-caruncular endometrium of sheep.

Ewe No.	Day of Cycle	6-oxo-$PGF_{1\alpha}$		
		Myometrium	Caruncular Endometrium	Non-caruncular Endometrium
Tissue Content (ng mg^{-1})				
76	3	0.097	0.005	0.005
89	5	0.032	0.032	0.085
105	8	0.050	0.017	0.070
111	13	0.063	0.042	0.142
87	15	0.021	0.039	0.040
88	15	0.014	0.104	0.119
94	16	0.066	0.004	0.220
Synthesizing Ability (ng mg^{-1} 90 min^{-1})				
76	3	0.53	0.13	0.19
89	5	0.23	0.18	0.54
105	8	0.17	0.19	0.13
111	13	0.08	0.16	0.13
87	15	0.61	0.75	0.65
88	15	0.28	0.34	0.22
94	16	0.62	0.34	0.57

There was no difference in $PGF_2\alpha$ and PGE_2 content or synthesizing ability between caruncular and non-caruncular endometrial tissue. However, $PGF_2\alpha$ content and synthesizing ability of myometrium was much lower than that for endometrium (see Fig. 10 and Table I).

The changes in 6-oxo-$PGF_1\alpha$ content and synthesizing ability of uterine myometrium and caruncular and non-caruncular endometrial tissue are shown in Table II. No signficant changes occurred in either 6-oxo-$PGF_1\alpha$ content or synthesizing ability of the three types of uterine tissue (see Fig. 10 and Table II).

DISCUSSION

The values for the content of $PGF_2\alpha$ in the uterine tissue found in the present experiment are comparable to those found by Pexton, Ford, Wilson, Butcher & Inskeep (1975). However, the $PGF_2\alpha$ content of caruncular and non-caruncular endometrial tissue appear at first sight to contradict the findings of Louis, Parry, Robinson, Thorburn & Challis (1977). They found that caruncles contained more $PGF_2\alpha$ and also produced more $PGF_2\alpha$ on incubation than did the intercaruncular tissue. However, the intercaruncular tissue used in their experiments consisted of endometrium and myometrium. As we, like Pexton *et al.* (1975), have found signficantly lower concentrations of $PGF_2\alpha$ in the myometrium, the difference observed by Louis *et al.* (1977) was

probably due to the inclusion of myometrium in their inter-caruncular tissue. The signficant increase of $PGF_2\alpha$ content towards the end of oestrous cycle suggests the involvement of this hormone in luteolysis.

6-oxo-$PGF_1\alpha$ was detected in caruncular, non-caruncular and myometrial tissue. The absence of any signficant changes in either the content and synthesizing ability of 6-oxo-$PGF_1\alpha$ and PGE_2 by these tissues indicates that these compounds are probably not involved in luteolysis.

EXPERIMENT III: Daily simultaneous measurement of uterine $PGF_2\alpha$ and PGE_2 content, synthesis and release.

Objective:

To take daily simultaneous measurements of the uterine content of $PGF_2\alpha$ and PGE_2, of the ability of the endometrial tissue to synthesize these prostaglandins and the concentration of $PGF_2\alpha$ in the uterine vein. The relationship between the measured values should indicate the area of prostaglandin sythesis at which extra-uterine factors act to cause the increase in the release of $PGF_2\alpha$ needed for luteolysis.

Animals and Methods

Four mature ewes (ewe 50, 76, 86 and 88) were used in this experiment. The animals were prepared and anaesthetized as described previously in Experiment I. However, in some animals the anaesthesia was induced with intravenous injection of 20 ml Saffan (alphaxalone & alphadolone acetate, Glaxo Laboratories Ltd., Greenford, Middlesex) instead of nitrous oxide and halothane.

The experimental procedure involved first the anastomosis of the utero-ovarian vein to the anterior mammary vein (as described in Experiment I) and then fistulation of the basal end of one uterine horn to the exterior. The fistulation was performed after the patency of the anastomosis had been checked by X-radiography (see Experiment I). In ewe 88 the anastomosis was also rechecked by X-radiography after injecting 5 ml radiopaque into

the uterine vein after laparatomy prior to fistulation.

Fistulation

After the re-establishment of normal oestrous cycles and the checking of the patency of the anastomosis, the base of the left uterine horn of all animals was fistulated to the exterior.

The uterus was located through a mid-ventral incision just to the left of the midline, and freed from any adhesions that were present. The left uterine horn was then ligated at the base and severed. The basal end of the left horn was then passed through a small hole in the abdominal wall just in front of the left segment of the udder and sutured in position (see Fig 11) with single interrupted sutures of nylon through all layers. A dressing and antibiotics (Streptopen Q.R. cerate, Glaxo Veterinary, Greenford, Middlesex) were applied to the fistula to prevent contamination and the dressing was changed daily.

Samples and Extraction

At each sampling two endometrial biopsies (each greater than 20 mg) were obtained, using a home-made stainless-steel biopsy instrument (Fig 12) and about 3 ml of uterine venous blood were taken by venipuncture from the anastomosed utero-mammary vein. Daily simultaneous samples of uterine tissue and venous blood were taken during the latter part (day 12 onwards) of a total seven

Figure 11

Photographs showing the position and appearance of the fistulated end of the uterine horn in ewe 86 (A & C) and 88 (B & D).

"After ligation and severence, the basal end of the left uterine horn was passed through a small hole in the abdominal wall just in front of the left segment of the udder and sutured in position with single interrupted sutures".

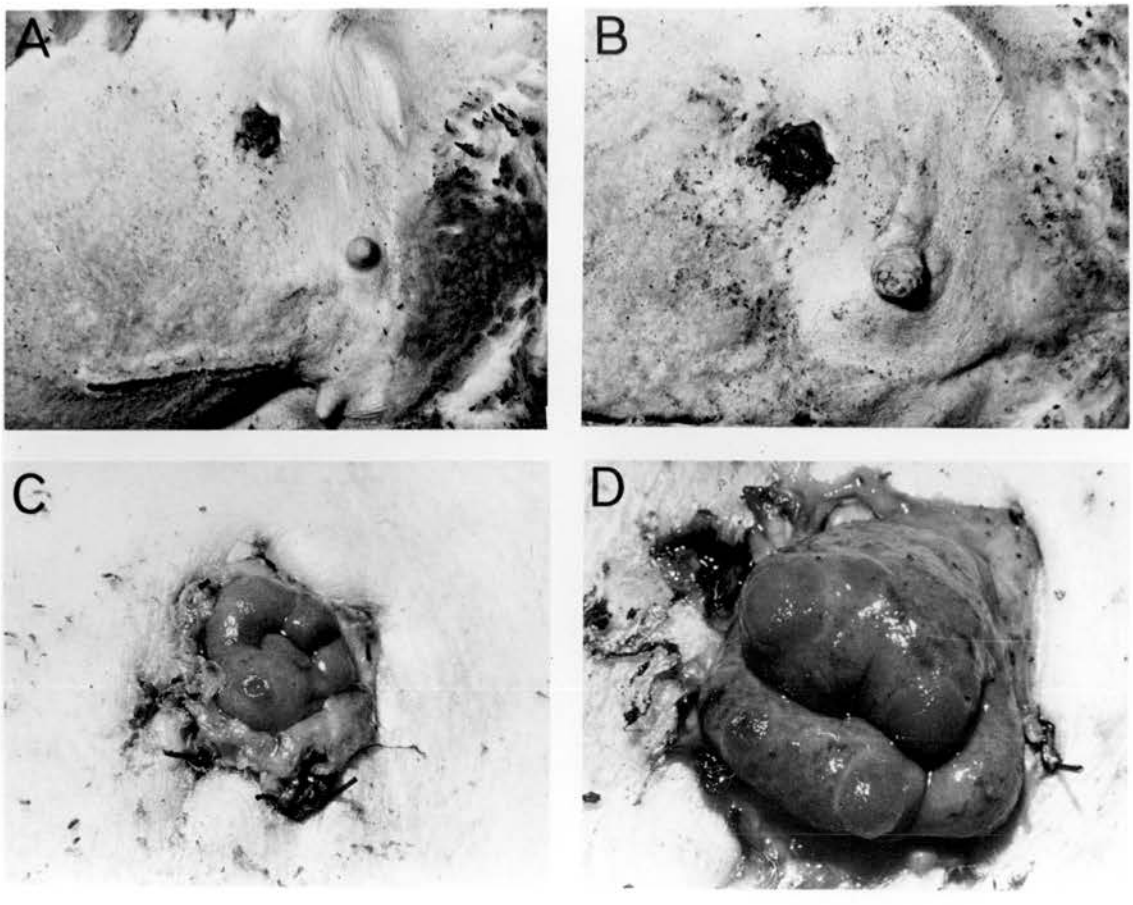
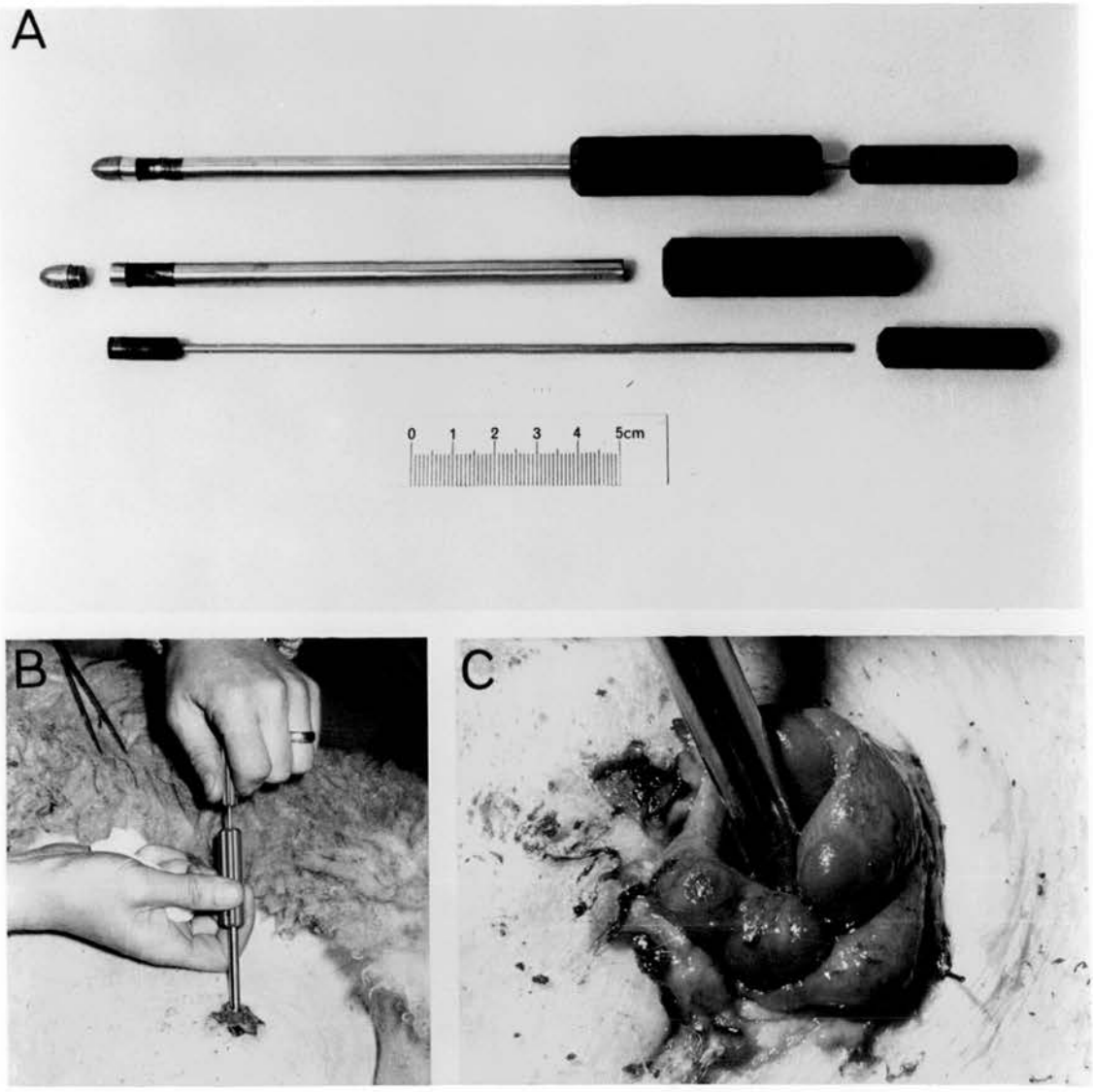

A
B
C
D

Figure 12

A - photograph of the home-made stainless-steel biopsy instrument showing it assembled (upper) and in pieces (lower); B & C - showing the instrument in use.


A
0 1 2 3 4 5cm
B
C

oestrous cycles from four ewes. Each endometrial biopsy was described by visual examination as either caruncle or non-caruncular tissue and weighed. The method of extraction used in these experiments were as previously described in Experiments I and II. Four simultaneous tissue samples were taken daily during both cycles (A & B) investigated in ewe 86. In the first cycle (ewe 86A) duplicate measurement of both $PGF_2\alpha$ and PGE_2 content and synthesizing ability were made to ascertain how paired samples varied. During the second cycle (ewe 86B) only duplicate incubations were performed in order to find whether the availability of arachidonic acid was a limiting factor in the tissue prostaglandin synthesis in vitro. Arachidonic acid (20 µg) in 20 µl ethyl alcohol was added to one homogenate while the solvent alone was added to the other homogenates.

Statistical Analysis

The significance of the difference between various days of the cycle and between the various tissues was checked by using Student's t-test for paired differences. The relationship between the various parameters measured was checked by calculating the correlation coefficients of the regression lines.

RESULTS/

RESULTS

The visual discrimination between caruncular and non-caruncular endometrial tissue taken by biopsy proved to be easier than had been anticipated. Based on all biopsy samples, no consistent differences could be found between $PGF_{2\alpha}$ and PGE_2 content and synthesizing ability of caruncular and non-caruncular endometrial tissue. Therefore, in the following results, no distinction will be made between the two types of endometrial tissue. In no instance was myometrial tissue retrieved at biopsy. This was confirmed by failure to find any damage to the myometrium of the fistulated uterine horn at post-mortem examination (see Fig 13)

Due to individual variation between animals and even between cycles in the same animals, all cycles are illustrated individually using the first day of oestrus as day 1. The length of oestrus itself varied from one to three days. Only cycles of normal length (16-20 days) and characteristics have been included in these results.

The changes in endometrial $PGF_{2\alpha}$ and PGE_2 content and synthesizing ability during the latter part of seven oestrous cycles are shown in Fig 14 and 15. Simultaneous plasma $PGF_{2\alpha}$ and progesterone concentrations were measured in all of these cycles (Fig 14). The plasma concentrations are equivalent to uterine venous concentrations as a patent utero-mammary vein anastomosis was present. Ewe 76 has very high plasma progest-

Figure 13

A photograph (A) and explanatory diagram (B) showing the fistulated uterine horn of ewe 88 opened longtudinally at post-mortem. 1 - normal caruncles; 2 - endometrium devoid of caruncles; 3 - myometrium stripped of endometrium; 4 - cut edge of normal myometrium; 5 - unopened part of uterine horn.

A

B

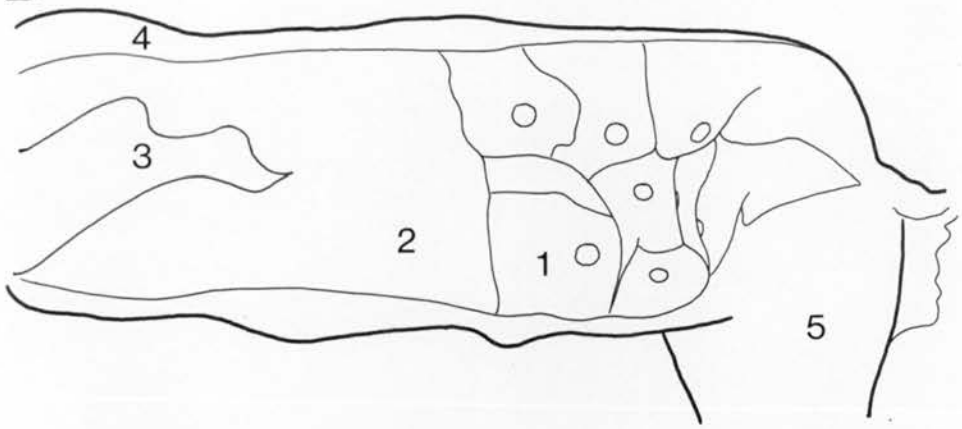


Figure 14

Diagram of the simultaneous concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$(hatched bars) in the anastomosed utero-mammary veins, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ (solid bars) and the synthesizing ability (ng mg^{-1} 90 min^{-1}) of endometrial tissue (open bars) during the latter part of seven individual oestrous cycles. The means and standard errors of the means (S.E.M.) of all these cycles are also shown. In cycle A of ewe 86 the range bars indicate the values of the duplicate samples and in cycle B of ewe 86 the range bars indicate the change in synthesizing ability caused by the addition of 20 µg arachidonic acid. In all ewes the ovary adjacent to the fistulated uterine horn had been removed. The shaded bars below the abscissa indicates the days of oestrus. The asterisks indicate that no sample was taken.

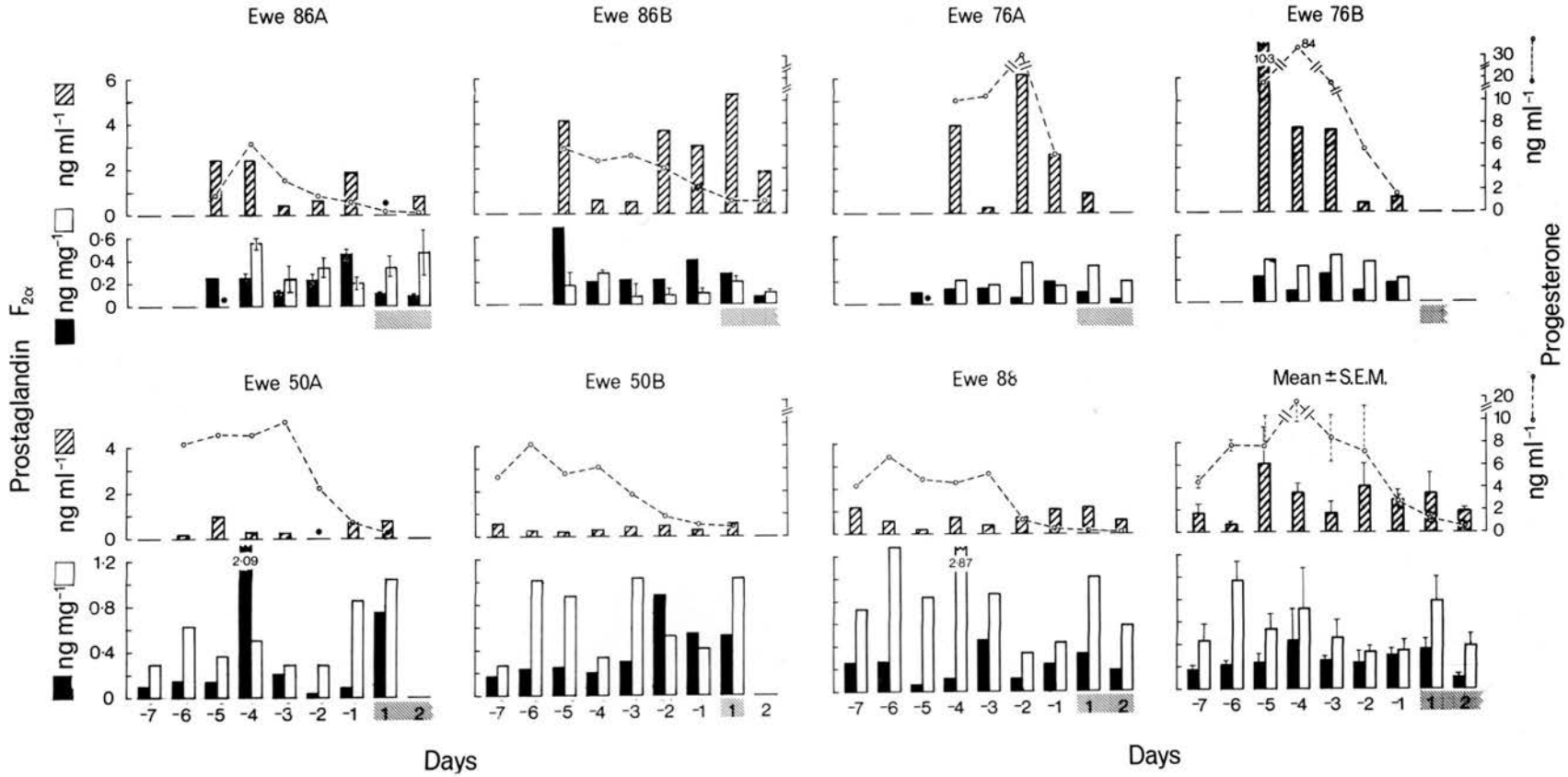
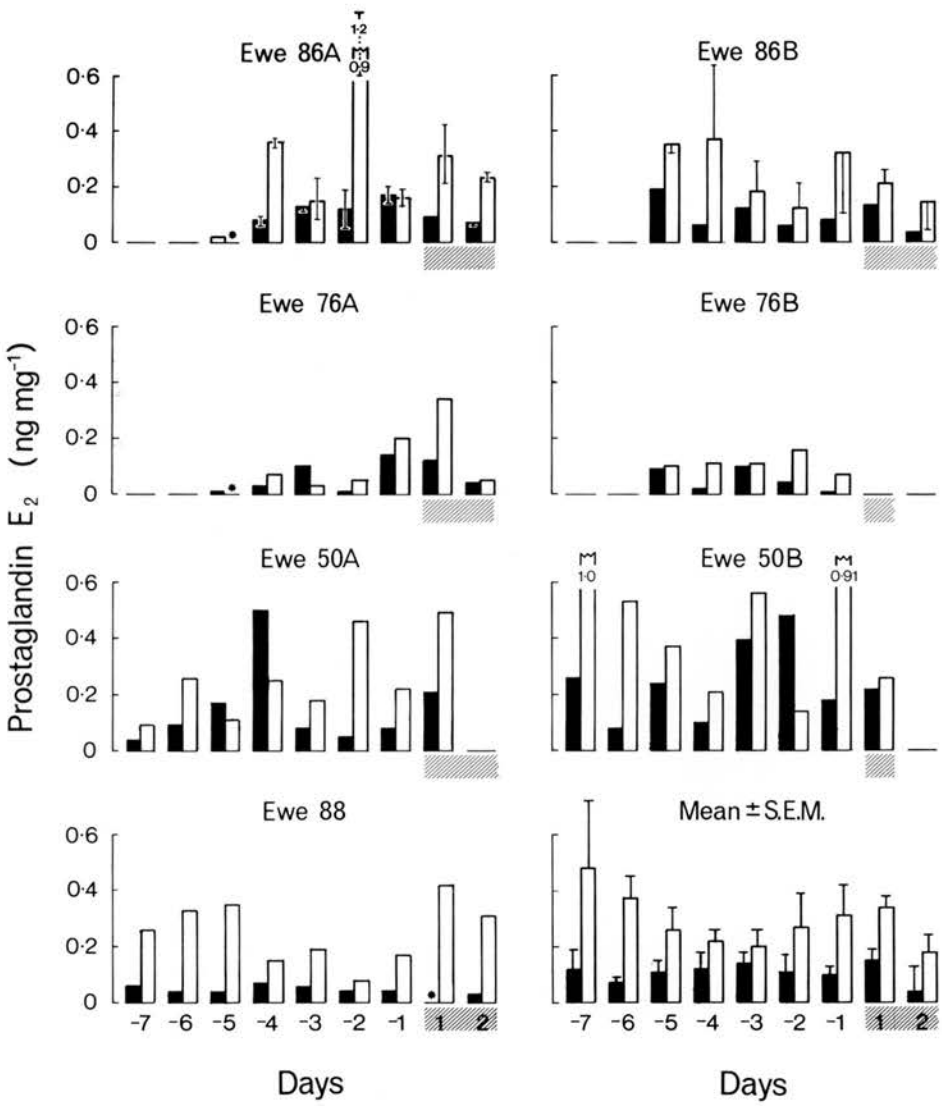

Ewe 86A
Ewe 86B
Ewe 76A
Ewe 76B
Ewe 50A
Ewe 50B
Ewe 88
Mean ± S.E.M.
Prostaglandin F2α
ng ml-1
ng mg-1
Progesterone
ng ml-1
2·09
2·87
1·03
·84
Days
Days

Figure 15

Diagram of the endometrial tissue content (ng ml^{-1}) of PGE_2 (solid bars) and the PGE_2 synthesizing ability (ng $mg^{-1}90\ min^{-1}$) of endometrial tissue (open bars) during the latter part of seven individual oestrous cycles. The means and standard errors of the means (S.E.M.) of all these cycles are also shown. In cycle A of ewe 86 the range bars indicate the values of duplicate samples and in cycle B of ewe 86 the range bars indicate the change in synthesizing ability caused by the addition of 20 µg arachidonic acid. In all ewes the ovary adjacent to the fistulated uterine horn had been removed. The shaded bars below the abscissa indicates the days of oestrus. The asterisks indicate that no sample was taken.


Ewe 86A
Ewe 86B
Ewe 76A
Ewe 76B
Ewe 50A
Ewe 50B
Ewe 88
Mean ± S.E.M.
Prostaglandin E_2 (ng mg^{-1})
0·6
0·4
0·2
0
-7 -6 -5 -4 -3 -2 -1 1 2
Days

erone concentrations which resulted in high values for the mean concentrations for this group of animals. Several groups of workers also reported considerable individual variation of progesterone concentration and its rate of decline at the end of oestrous cycle (Bassett et al. 1969; Fylling, 1970; Pant et al. 1977). This variation may be more apparent than real in view of the fluctuation in uterine vein progesterone seen in Experiment I. Nevertheless progesterone concentration in all animals fell normally at the end of the cycle indicating that luteal regression had taken place.

As would be expected, plasma concentrations of $PGF_2\alpha$ were occasionally elevated in the uterine venous blood, however these were not consistently accompanied by changes in either the uterine content or synthesizing ability. Also there was no significant changes in endometrial $PGF_2\alpha$ or PGE_2 content but, $PGF_2\alpha$ synthesizing ability was significantly higher on day 1 than on day -1 ($p<0.05$) of the oestrous cycle. There was also no significant differences in endometrial PGE_2 synthesizing ability. Consequently, there was no relationship between the ability of the endometrium to sythesize $PGF_2\alpha$ and PGE_2. However, there was a direct relationship between the endometrial content of $PGF_2\alpha$ and PGE_2, although no relationship existed between endometrial content of $PGF_2\alpha$ or PGE_2 and the ability of this tissue to synthesize either of these prostaglandins. Likewise, changes in the PGE_2 content of the endometrium were not related to changes in PGE_2 synthesizing ability and similarly with $PGF_2\alpha$.

The values for duplicate tissue samples obtained in ewe 86A showed relatively small differences for measurements of prostaglandins content (mean % differences; $PGF_2\alpha$ 26.1%; PGE_2 , 26.5%). However, larger differences were found for duplicate incubations (mean % differences; $PGF_2\alpha$, 44.3%; PGE_2 36.2%). Also the addition of 20µg arachidonic acid to one incubate in ewe 86B made no significant differences to the amount of $PGF_2\alpha$ or PGE_2 synthesized.

DISCUSSION

The removal of a biopsy occasionally caused some slight bleeding from the endometrium, but this was temporary and stopped within 30-60 seconds. The uterine fistula proved to be more resistant to infection than had been expected. However, slight infection in the fistula occurred on one occasion but the data from this cycle have not been included in the results. All tissue samples were transferred quickly to either alcohol or Tyrodes solution. It is unlikely, therefore, that prostaglandin production was appreciably altered by the trauma of removing the samples or by inflammation of the endometrium.

The values for the content of $PGF_2\alpha$ in uterine caruncular and non-caruncular endometrial tissue found in the present experiment are comparable to those in Experiment II and those found

by Wilson, Cenedella, Butcher & Inskeep (1972) and Lewis & co-workers (1977, 1978). Again no difference was found between the $PGF_{2\alpha}$ and PGE_2 content and synthesizing ability of caruncular and non-caruncular endometrial tissue.

Surprisingly no relationship emerged between endometrial $PGF_{2\alpha}$ content, synthesizing ability and secretion. The finding that endometrial $PGF_{2\alpha}$ synthesizing ability was signficantly higher in day 1 than at one day before the onset of oestrus was unexpected. At this time oestradiol levels in plasma are high which can effect the release of $PGF_{2\alpha}$ and LH from the brain (see p.85). Thus as oestradiol is known to increase the synthesizing ability of the uterus for $PGF_{2\alpha}$ in several species (see General Discussion), it is possible that the high level of circulatory oestradiol at this time is sufficient to increase the $PGF_{2\alpha}$ synthesizing capacity in the contralateral uterine horn of the unilaterally ovariectomized ewes. The direct relationship between the endometrial contect of $PGF_{2\alpha}$ and PGE_2 suggests either that PGE_2 production is a by-product of $PGF_{2\alpha}$ production or that in both instances it is the availability of a common precursor that controls the tissue content of the two prostaglandins. The failure of arachidonic acid to significantly alter the amount of $PGF_{2\alpha}$ or PGE_2 synthesized in ewe 86B indicates that the availability of this precursor was not a limiting factor in the synthesis of $PGF_{2\alpha}$ and PGE_2 in this incubation system. This is in keeping with the very recent results of Smith, Husling & Fogwell (1979), who measured prostaglandin-forming cyclo-oxygenase activity in ovine uterine microsomes. An increase in the activity of this enzyme between day 13-15 led them to suggest that the increased $PGF_{2\alpha}$ released and hence the onset of luteolysis depended on an increase in the efficiency with which arachidonate was converted to $PGF_{2\alpha}$ - precursor rather than substrate availability. However, their finding that cyclo-oxygenase activity only

increased in caruncular tissue and not in intercaruncular tissue is difficult to reconcile with our finding that $PGF_2\alpha$ levels are the same in these two types of endometrium.

No relationship was found between content, secretion and sythesizing ability of $PGF_2\alpha$ when daily samples were taken during the oestrous cycle from a uterine horn from which the adjacent ovary was absent. The lack of any relationship may be due to one of the following reasons:

1. The frequency of sampling may be too infrequent to see any relationship.

2. The absence of the ovary adjacent to the fistulated uterine horn may be of importance.

Thus in the following experiments these two factors will be considered.

EXPERIMENT IV: More frequent measurement of uterine $PGF_{2\alpha}$ and PGE_2 content, synthesis and release.

Objective:

In the previous experiments no relationship was found to exist between uterine endometrial $PGF_{2\alpha}$ content, synthesizing ability and secretion when daily samples were taken in the absence of an adjacent ovary; perhaps more frequency sampling is required for a relationship to emerge.

Animals and Samples

Four mature ewes were used in the present experiment (ewe 76, 77, 86 and 88). All the animals included in this experiment have a patent utero-mammary vein anastomosis, the left uterine horn fistulated to the exterior and the left ovary removed (as described previously in Exp. III).

Various frequencies of sampling were adopted in the current experiment within the limit of what it was considered the animal could tolerate without deviating from normal (see table III).

At each sampling in the current experiment two endometrial biopsies (each greater than 20 mg) were obtained and about 3 ml of uterine venous blood were taken from the anastomosed utero-mammary vein. Sampling was continued throughout the 24 hr period except for the omission of one sampling in the middle of the night.

Table III: The frequencies of sampling during various days of oestrous cycle in four ewes as adopted in Exp. IV.

Ewe No.	Day of the cycle on which samples taken	Frequency of Sampling	
		Blood	Tissue
77A	12-18	3 hr	Daily
77B	11-13*	3 hr	Daily
88A	12-16†	3 hr	6 hr
88B	12-14	3 hr	6 hr
86	15-16	3 hr	3 hr††
76	12-13	½ hr	4 hr

* Daily tissue and blood samples also taken from day 4 to 10.

† Daily tissue and blood samples also taken from day 17 to day 2 of the next cycle.

†† Duplicate tissue samples for incubation were taken from ewe 86.

The methods used for collection and extraction of the samples are the same as those described in Exp. I & III. Daily samples of both blood and tissue were also taken from cycle B of ewe 77 from day 4-10 and from cycle A of ewe 88 from day 17 to day 2 of the next cycle. In ewe 86 duplicate tissue samples for incubation were taken throughout the experimental period. To one of the duplicate incubates

20 µg of arachidonic acid in 20 µl of ethyl alcohol was added while the solvent alone was added to the other.

RESULTS

In all animals used in the present experiment the anastomosis was found to be patent after checking by X-radiography. Thus as the blood samples were taken from anastomosed uteromammary vein, the blood concentrations of $PGF_2\alpha$ and progesterone on the selected days of the six oestrous cycles studied are those of uterine venous blood.

The concentrations of progesterone and $PGF_2\alpha$ in uterine venous plasma and the endometrial tissue content and synthesizing ability of $PGF_2\alpha$ and PGE_2 for the six cycles are illustrated individually in Figs 16-21. Ewe 77 was sampled in what turned out to be the last two cycles of the season. The ewe had a long 24-day cycle for cycle A (Fig 16), while the animal did not return to oestrus in cycle B (Fig 17) due to the onset of the summer "anoestrus". All the other animals showed normal 16-18 days oestrous cycle (Figs 18-21).

Statistical analysis of the result for relationships between the different parameters in simultaneous samples showed no significant relationships except in three instances, i.e. endometrial synthesizing ability of $PGF_2\alpha$ and that of PGE_2 ($p<0.001$) in ewe 88A (Fig 18); the endometrial content of $PGF_2\alpha$ and its synthesizing ability ($p<0.05$) in ewe 88B (Fig 19) and the plasma $PGF_2\alpha$ and the endometrial content of $PGF_2\alpha$

Figure 16

Diagram of the concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$(solid circles) in the anastomosed utero-mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the synthesizing ability (ng $mg^{-1}90\ min^{-1}$) of endometrial tissue (open bars) from cycle A of ewe 77. The utero-mammary venous blood was collected every 3 hr on days 12 to 18 while daily uterine endometrial biopsies were taken at 10.00 hr during the same period. In this ewe the ovary adjacent to the fistulated uterine horn had been removed and the animal had 24-day oestrous cycle.

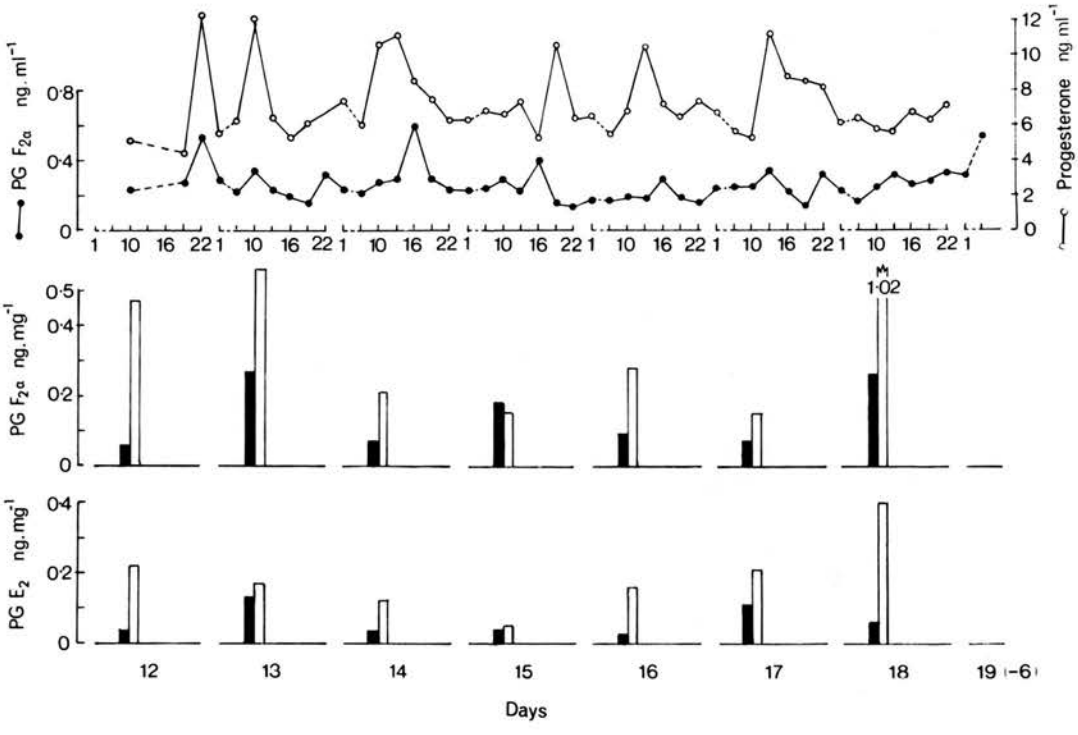

PG $F_{2\alpha}$ ng.ml^{-1}
0·8
0·4
0
Progesterone ng ml^{-1}
12
10
8
6
4
2
0
1 10 16 22 1 10 16 22 1 10 16 22 1 10 16 22 1 10 16 22 1 10 16 22 1
PG $F_{2\alpha}$ ng.mg^{-1}
0·5
0·4
0·2
0
M
1·02
PG E_2 ng.mg^{-1}
0·4
0·2
0
12
13
14
15
16
17
18
19 1-6
Days

Figure 17

Diagram of the concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$ (solid circles) in the anastomosed utero-mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the synthesizing ability (ng $mg^{-1}90$ min^{-1}) of endometrial tissue (open bars) from cycle B of ewe 77. Daily simultaneous utero-mammary venous blood and endometrial biopsies were collected on days 4 to 10 of the oestrous cycle and then 3-hourly blood samples and daily biopsies were taken for the next three days of the same cycle. In this ewe the ovary adjacent to the fistulated uterine horn had been removed and the animal did not return to oestrus due to the intervention of the summer "anoestrus".

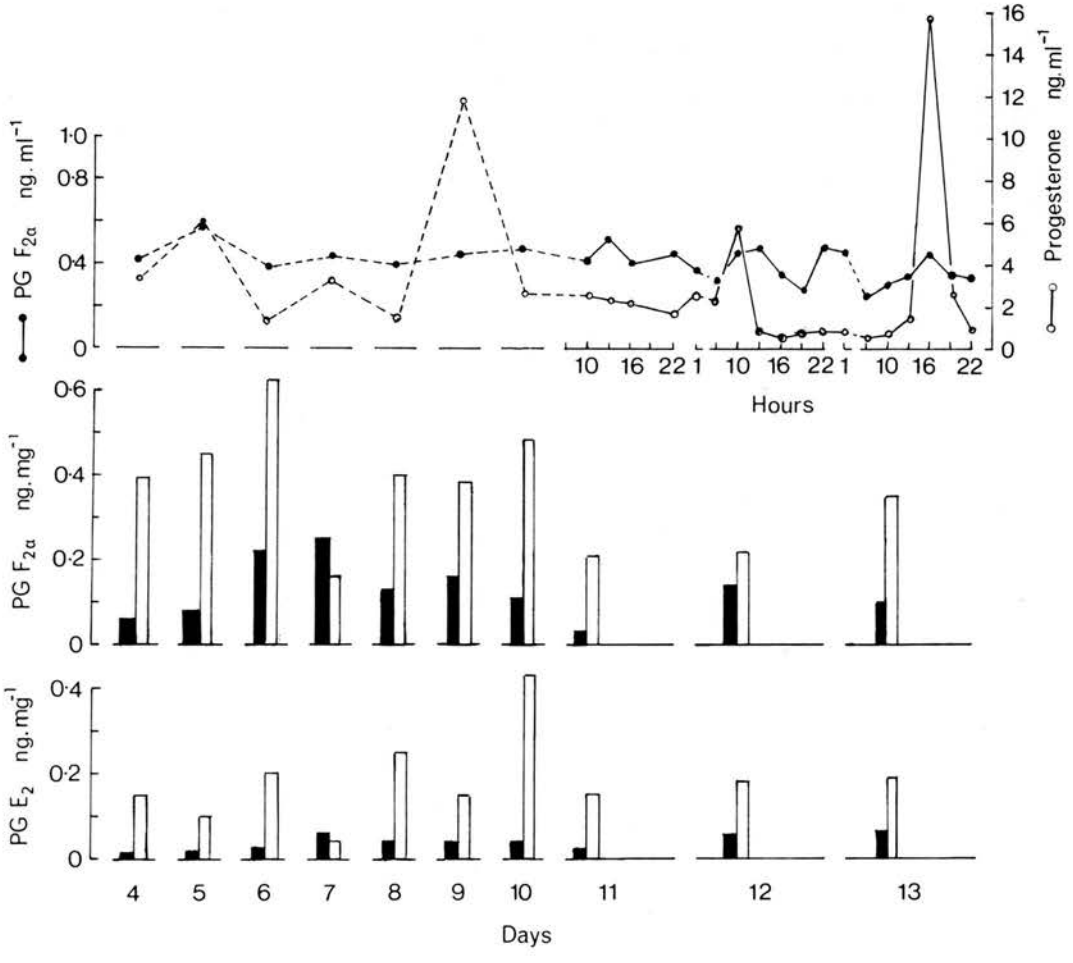

PG $F_{2\alpha}$ ng.ml^{-1}
1·0
0·8
0·4
0
Progesterone ng.ml^{-1}
16
14
12
10
8
6
4
2
0
10 16 22 1 10 16 22 1 10 16 22
Hours
PG $F_{2\alpha}$ ng.mg^{-1}
0·6
0·4
0·2
0
PG E_2 ng.mg^{-1}
0·4
0·2
0
4 5 6 7 8 9 10 11 12 13
Days

Figure 18

Diagram of the concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$ (solid circles) in the anastomosed utero-mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the synthesizing ability (ng $mg^{-1}90\ min^{-1}$) of endometrial tissue (open bars) from cycle A of ewe 88. Utero-mammary venous blood (every 3 hr) and biopsies (every 6 hr) were collected on day 12-16 of the oestrous cycle. Daily simultaneous venous blood and biopsies were also collected at 10.00 hr from day 17 to the second day of the next cycle. In this animal the ovary adjacent to the fistulated uterine horn had been removed and the animal had a normal 18-day cycle.

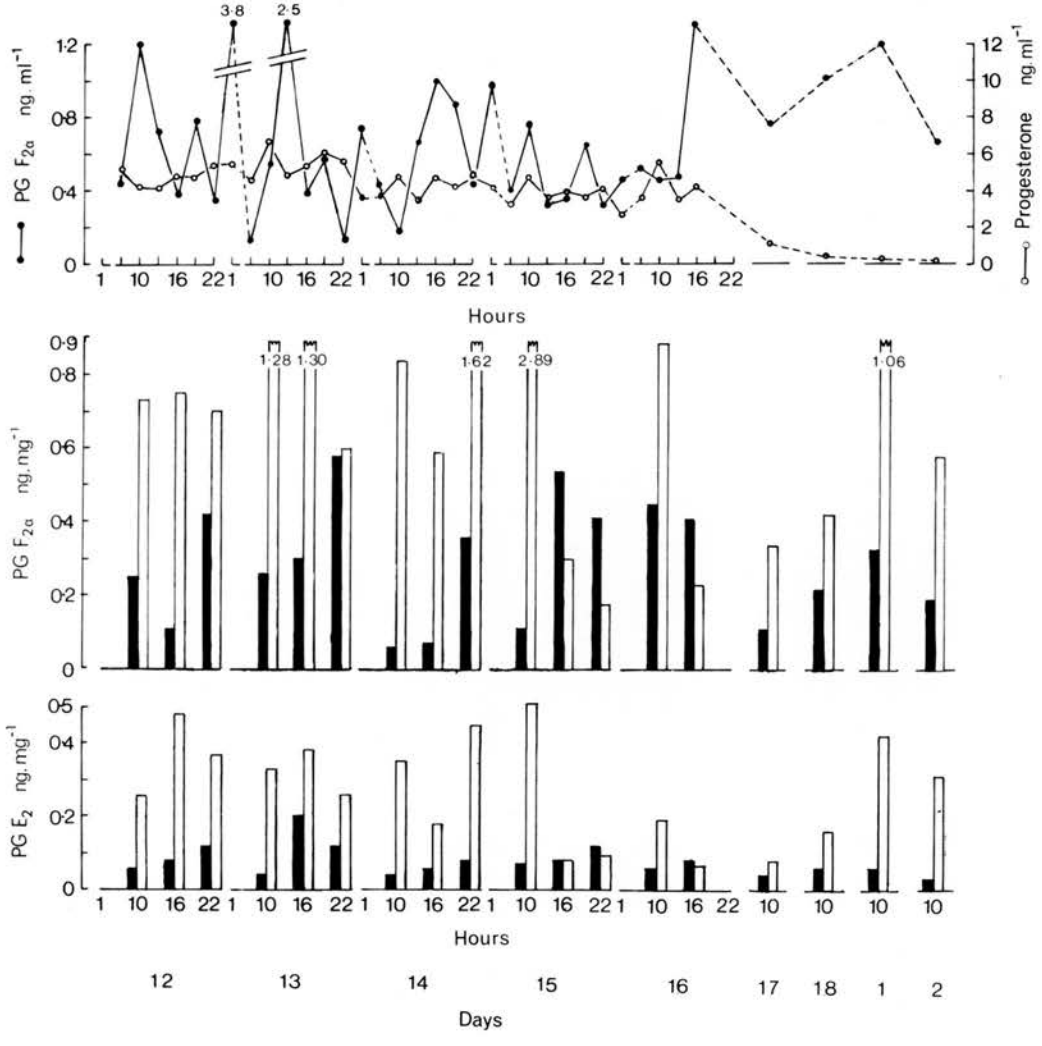

3·8
2·5
PG F2α ng.ml-1
Progesterone ng.ml-1
Hours
1·28
1·30
1·62
2·89
1·06
PG F2α ng.mg-1
PG E2 ng.mg-1
Hours
12
13
14
15
16
17
18
1
2
Days

Figure 19

Diagram of the concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$ (solid circles) in the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the synthesizing ability (ng $mg^{-1}90\ min^{-1}$) of endometrial tissue (open bars) from cycle B of ewe 88. Uteromammary venous blood was taken at 3 hr intervals on day 12, 13 and 14 of the oestrous cycle. Endometrial biopsies at 6 hourly intervals were also taken during the same period of the cycle. In this animal the ovary adjacent to the fistulated uterine horn had been removed and the animal had a normal 17-day cycle.

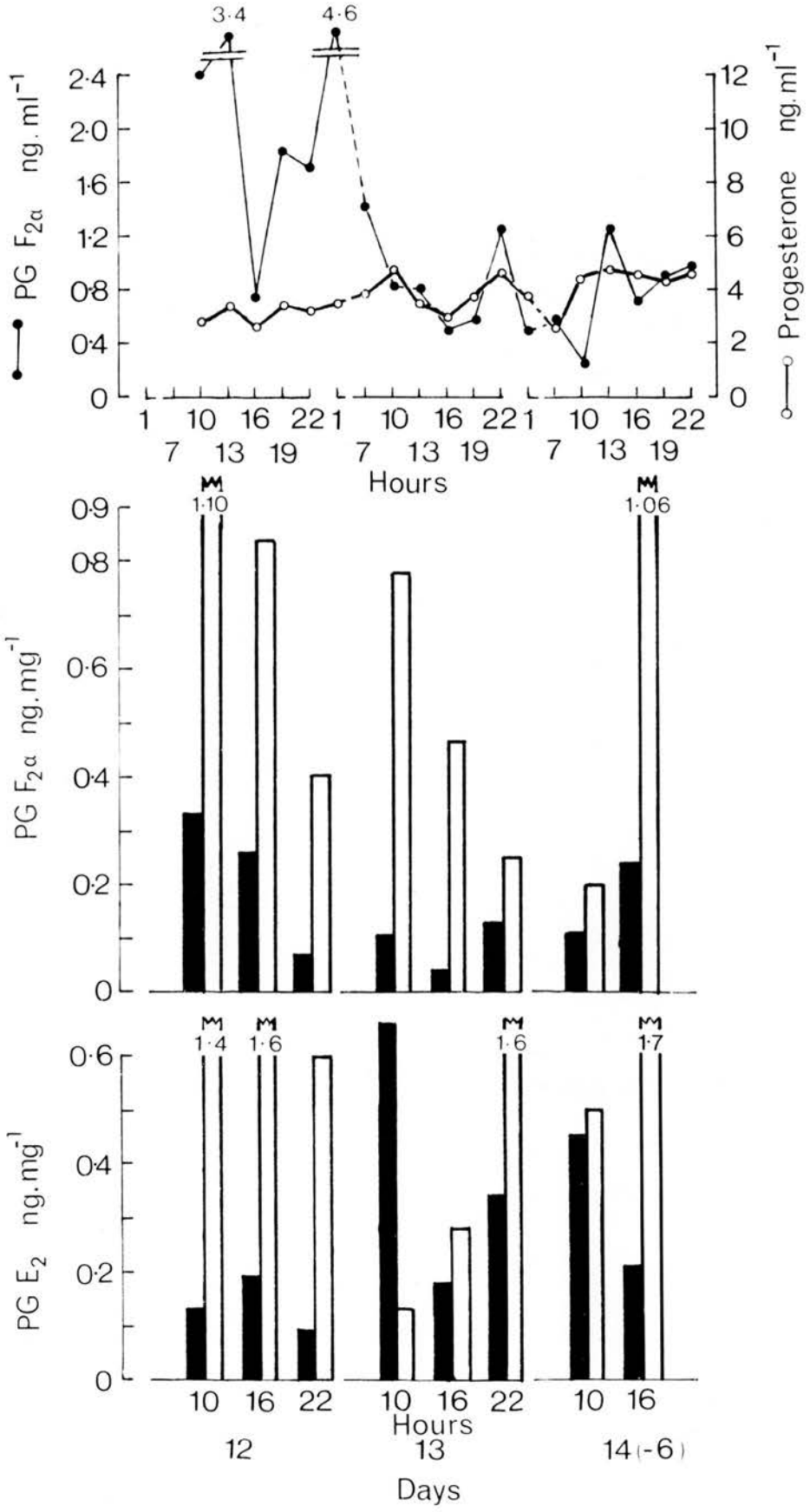

3·4
4·6
PG $F_{2\alpha}$ ng.ml^{-1}
Progesterone ng.ml^{-1}
2·4
2·0
1·6
1·2
0·8
0·4
0
12
10
8
6
4
2
0
1 10 16 22 1 10 16 22 1 10 16 22
7 13 19 7 13 19 7 13 19
Hours
1·10
1·06
PG $F_{2\alpha}$ ng.mg^{-1}
0·9
0·8
0·6
0·4
0·2
0
1·4
1·6
1·6
1·7
PG E_2 ng.mg^{-1}
0·6
0·4
0·2
0
10 16 22 10 16 22 10 16
Hours
12
13
14 (-6)
Days

Figure 20

Diagram of the concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_{2\alpha}$ (solid circles) in the anastomosed utero-mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_{2\alpha}$ and PGE_2 (solid bars) and the sythesizing ability (ng mg^{-1} 90 min^{-1}) of endometrial tissue (open bars) from ewe 86. Simultaneous utero-mammary venous blood and uterine biopsies were collected in 3 hr intervals on day 15 and 16 of oestrous cycle. The range bars indicate the changes in synthesizing ability caused by the addition of 20 µg arachidonic acid. In this animal the ovary adjacent to the fistulated uterine horn had been removed and the animal had a normal 17-day cycle.

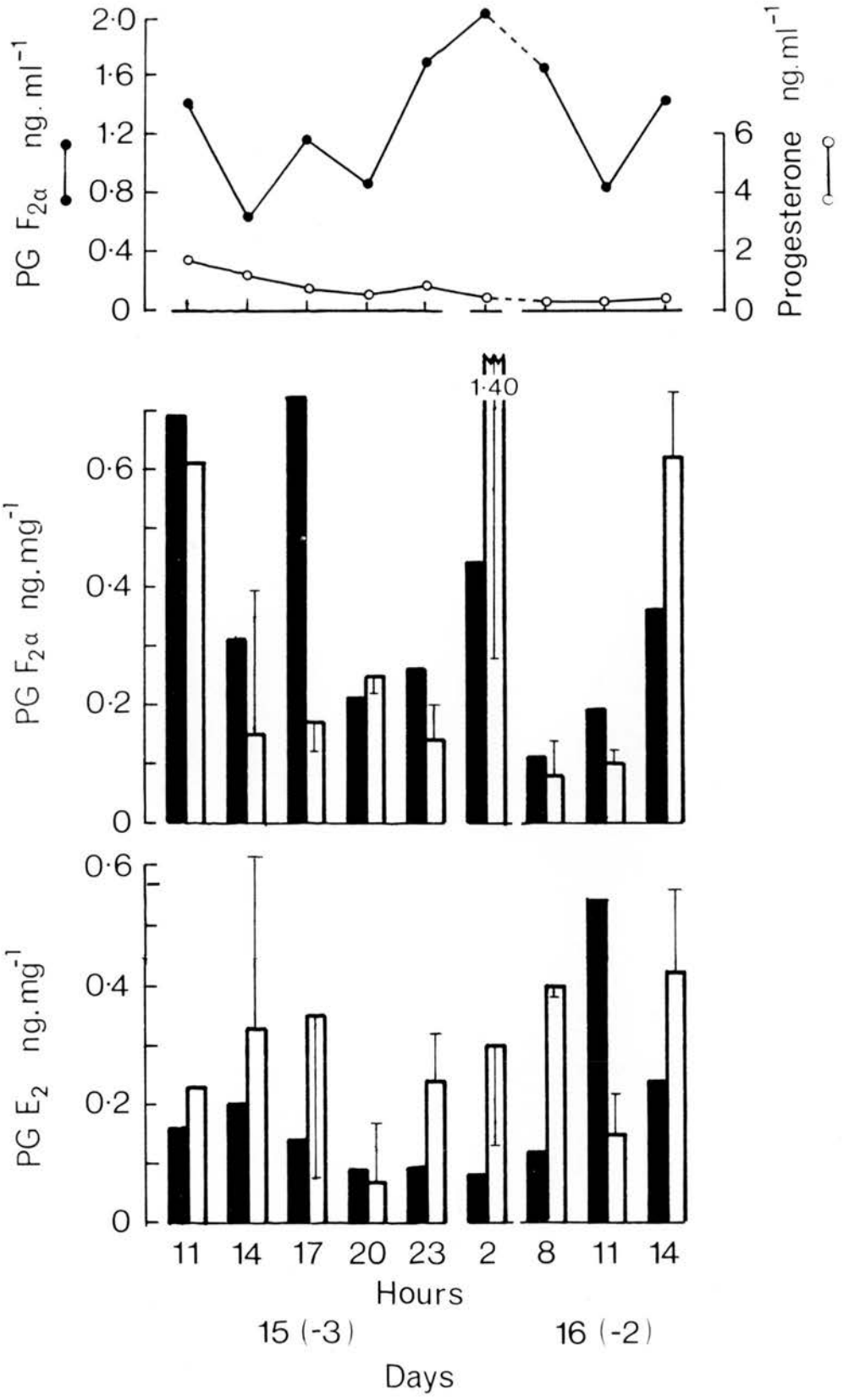
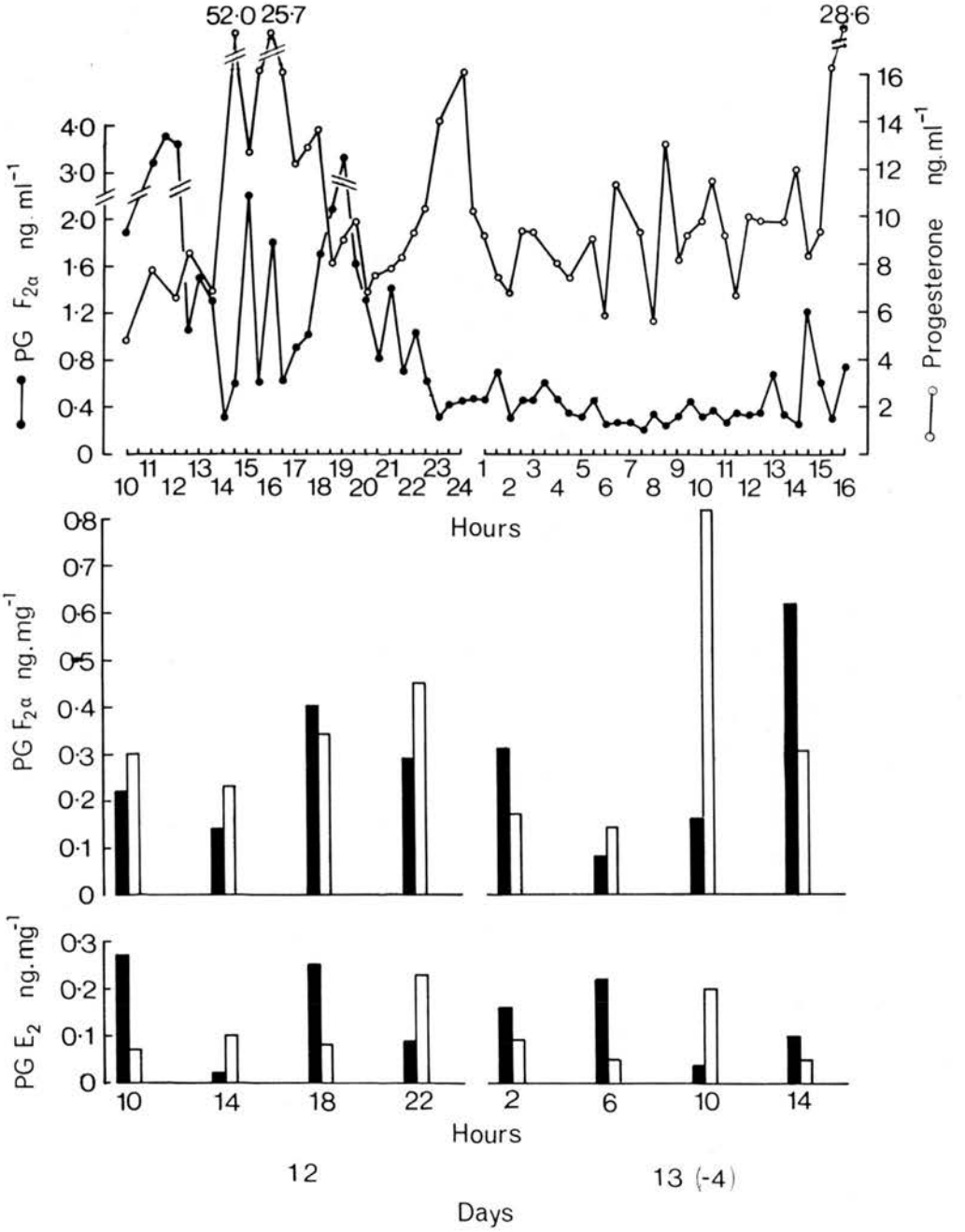

PG $F_{2\alpha}$ ng.ml^{-1}
2·0
1·6
1·2
0·8
0·4
0
6
4
2
0
Progesterone ng.ml^{-1}
1·40
PG $F_{2\alpha}$ ng.mg^{-1}
0·6
0·4
0·2
0
PG E_2 ng.mg^{-1}
0·6
0·4
0·2
0
11 14 17 20 23 2 8 11 14
Hours
15 (-3)
16 (-2)
Days

Figure 21

Diagram of the concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$ (solid circles) in the anastomosed utero-mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the synthesizing ability (ng $mg^{-1}90\ min^{-1}$) of endometrial tissue (open bars) from ewe 76. Throughout day 12 and 13 of oestrous cycle, samples of utero-mammary venous blood were taken every half-hour and uterine endometrial biopsies were taken every 4 hr. In this animal the ovary adjacent to the fistulated uterine horn had been removed and the animal had a normal 16-day cycle.


52·0 25·7
28·6
PG F2α ng.ml-1
Progesterone ng.ml-1
Hours
PG F2α ng.mg-1
PG E2 ng.mg-1
Hours
12
13 (-4)
Days

($p<0.05$) in ewe 76 (Fig. 21). No relationship were found between the different parameters and asynchronous plasma samples. However, a significant direct ($p<0.05$) relationship was found between the plasma concentration of progesterone and $PGF_2\alpha$ when $PGF_2\alpha$ level was compared with the progesterone level half-hour previously in ewe 76 (Fig. 21) between 10.00 - 20.00 hr on day 12. As in Exp. III, the addition of 20 µg arachidonic acid to one incubate in ewe 86 (Fig. 20) made no significant differences to the amount of $PGF_2\alpha$ or PGE_2 synthesized.

DISCUSSION

In the current experiments, the frequencies of sampling were increased within the limit of what it was considered that the animal could tolerate without deviating from normal. For instance, account was taken of the number of caruncles in a uterine horn and the amount of endometrial tissue that can be removed from the uterus without effecting the normal oestrous cycle. Ewe 77 was sampled at the last two cycles of the season, the extension of cycle A to 24 days is probably due to this fact as ewes 76, 86 and 88 had cycles of normal length after similar experimental treatments.

The values of the content of $PGF_2\alpha$ in uterine caruncular and non-caruncular endometrial tissue found in the present experiment are comparable to those in Exp. II, III and those found by Wilson, Cenedella, Butcher & Inskeep (1972) and Lewis and co-workers (1977, 1978). Also no difference was found between the $PGF_2\alpha$ and PGE_2 content and synthesizing ability of the two types of endometrial tissue.

The statistically significant relationships which emerged between the endometrial synthesizing ability of $PGF_{2\alpha}$ and that of PGE_2 in ewe 88A, the endometrial content of $PGF_{2\alpha}$ and its synthesizing ability in ewe 88B and the plasma $PGF_{2\alpha}$ and its endometrial content in ewe 76 are puzzling in view of their inconsistent occurrence. Nor can the general absence of relationship between plasma concentration of $PGF_{2\alpha}$ and the other parameters be explained on the basis of some delay at the secretory stage as plasma samples taken both before and after measurement of the prostaglandin content and synthesizing ability still failed to show any significant relationships. However, the significant relationship that emerged between the plasma concentration of progesterone and that of $PGF_{2\alpha}$ when $PGF_{2\alpha}$ level was compared with progesterone level half-hour previously in ewe 76 indicated that the increased $PGF_{2\alpha}$ release probably occurred in response to an increase in progesterone secretion at least in the period between 10.00 - 20.00 hr on day 12. However, the small peaks of progesterone on day 13 did not stimulate $PGF_{2\alpha}$ release.

The failure of arachidonic acid to significantly alter the $PGF_{2\alpha}$ or PGE_2 synthesizing ability in ewe 86 in 3 hr samples confirm the findings in Exp. III and indicates that indeed the availability of this precursor was not a limiting factor in the synthesis of $PGF_{2\alpha}$ or PGE_2 in this incubation system.

It is concluded that the infrequency of sampling is not a major factor contributing to failure to find any significant relationship between the various parameters in Exp. III.

EXPERIMENT V(A): Daily simultaneous measurement of uterine $PGF_2\alpha$ and PGE_2 content and synthesis with adjacent ovary *in situ*.

Objective :

As the previous experiments yielded no consistent relationships between uterine endometrial $PGF_2\alpha$ content, synthesizing ability and secretion in the absence of an adjacent ovary, it may be the absence of the ovary that is giving rise to this lack of relationships. Thus the present experiment was conducted to look at the situation when the adjacent ovary was present.

Anastomosis of the utero-ovarian and mammary veins by the method described by Thorburn & Mattner (1971) should prevent luteolysin from reaching the corpora lutea in the adjacent ovary (see p.100) and so lead to maintenance of the CL. Thus in the current experiment no attempt was made to anastomose the utero-ovarian vein from the fistulated horn. The secretion of $PGF_2\alpha$ from the fistulated horn is thus unknown in the present experiment.

Animals and Methods

The three animals used in the present experiment consisted of:

a) Two animals (ewe 67 and 89) in which anastomosis was attempted and unilateral ovariectomy was performed

as for Exp. III, but it was the uterine horn on the un-operated side that was fistulated to the exterior.

b) One animal (ewe 73) in which fistulation of one uterine horn was performed without previously removing an ovary or attempting a utero-mammary anastomosis.

From these three animals, daily endometrial biopsies were taken during the latter part of six oestrous cycles. In all cases an ovary remained attached to the fistulated uterine horn. Peripheral blood plasma was obtained daily during only 4 cycles (one cycle from ewe 67; three cycles from ewe 89). It was obtained by simple venipuncture from the unanastomosed mammary vein. The methods of extraction for the samples collected during this experiment were as explained previously in Exps. I and III.

RESULTS

Unfortunately, in the two animals used in the present experiment (ewe 67 and 89) the anastomosis was found not to be patent, while in the third animal (ewe 73) the anastomosis was not attempted. Thus the blood concentrations of the different components in the 4 cycles in which they were studied are equivalent to peripheral levels as it was taken from an un-anastomosed mammary vein. The results of the 6 oestrous cycles

studied in the present experiment are illustrated in Figs 22 and 23. As expected the peripheral plasma $PGF_2\alpha$ concentrations were low and without any large fluctuations. The progesterone concentration in peripheral plasma were lower and more stable than those in Exp. III, but fell normally at the end of the cycles indicating luteal regression. There was no significant changes in endometrial $PGF_2\alpha$ content, but $PGF_2\alpha$ synthesizing ability increased towards the end of the cycle and was significantly higher on day -3($p<0.02$) and -2($p<0.05$). There were no significant changes in endometrial PGE_2 synthesizing ability or PGE_2 content in this group of animals. There was also no relationship between the ability of endometrium to synthesis $PGF_2\alpha$ and PGE_2 but again there was a direct relationship between the endometrial content of $PGF_2\alpha$ and the content of PGE_2. No relationship existed between the endometrial content of $PGF_2\alpha$ and the ability of the endometrium to synthesize PGE_2 and vice versa. Thus the changes in $PGF_2\alpha$ content of the endometrium were apparently not related to that of synthesizing ability for $PGF_2\alpha$.

DISCUSSION

Again the values for the content of $PGF_2\alpha$ in uterine endometrial tissue found in the present experiment are comparable to those found in the previous experiments. In this group of animals there was no significant increase in endometrial $PGF_2\alpha$ and PGE_2 content over the period studied. However, endometrial

Figure 22

Diagram of the simultaneous concentration (ng ml^{-1}) of progesterone (open circle) and prostaglandin $F_2\alpha$ (hatched bars) in the mammary veins, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ (solid bars) and the sythesizing ability (ng mg^{-1} 90 min^{-1}) of endometrial tissue (open bars) during the latter part of the oestrous cycles. The means and standard error of the means (S.E.M.) for these cycles are also shown. In all ewes the ovary adjacent to the fistulated uterine horn was present. The shaded bars below the abscissa indicates the days of oestrus. The asterisk indicates that no sample was taken.

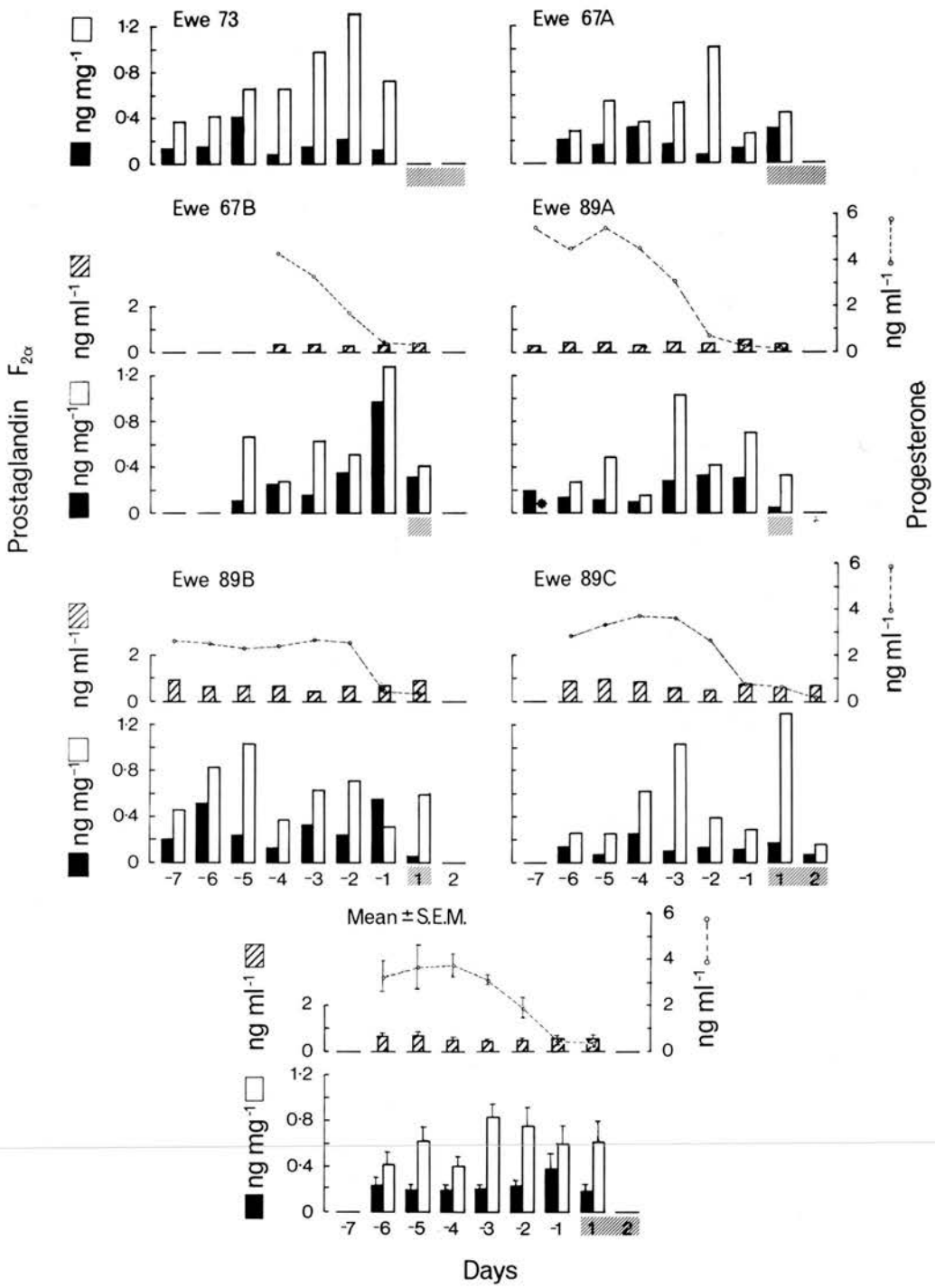
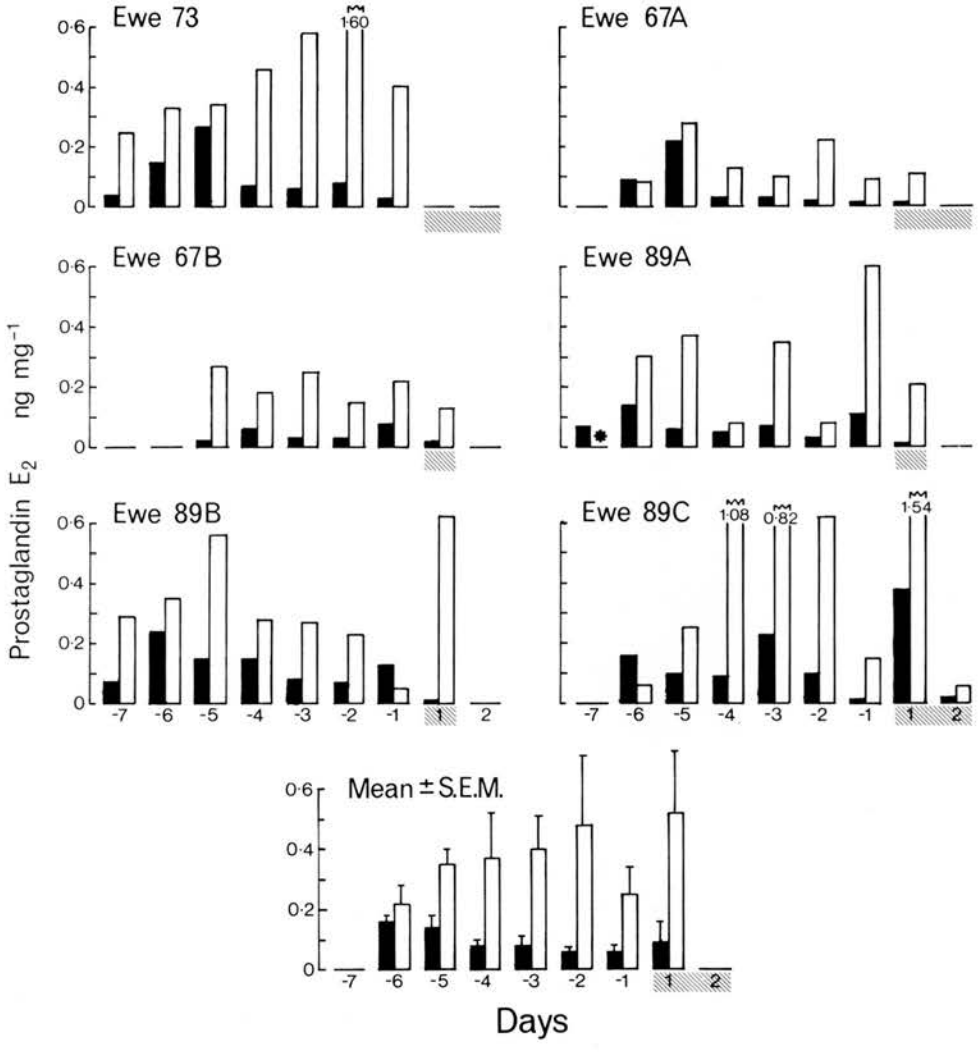

Ewe 73
Ewe 67A
Ewe 67B
Ewe 89A
Ewe 89B
Ewe 89C
Mean ± S.E.M.
Prostaglandin F2α
ng mg-1
ng ml-1
Progesterone
Days
-7 -6 -5 -4 -3 -2 -1 1 2

Figure 23

Diagram of the endometrial tissue content (ng ml^{-1}) of PGE_2 (solid bars) and the synthesizing ability (ng $mg^{-1}90\ min^{-1}$) of endometrial tissue (open bars) during the latter part of six individual oestrous cycles. The means and standard errors of the means (S.E.M.) for all these cycles are also shown. In all ewes the ovary adjacent to the fistulated uterine horn was present. The shaded bars below the abscissa indicates the days of oestrus. The astrisk indicates that no sample was taken.


Ewe 73
1·60
Ewe 67A
Ewe 67B
Ewe 89A
Ewe 89B
Ewe 89C
1·08
0·82
1·54
Mean ± S.E.M.
0·6
0·4
0·2
0
-7
-6
-5
-4
-3
-2
-1
1
2
Prostaglandin E_2 ng mg^{-1}
Days

$PGF_2\alpha$ synthesizing ability was signficantly higher three and two days before the onset of oestrus. Unfortunately, simultaneous measurement of $PGF_2\alpha$ concentration in the uterine venous plasma were not possible in this group. The increase in $PGF_2\alpha$ synthesizing ability of uterus on day -3 coincided with commencement of the fall in plasma progesterone concentrations, and confirms the involvement of $PGF_2\alpha$ in luteolysis. Smith, Husling & Fogwell (1979) have found an increase in prostaglandin-forming cyclo-oxygenase at this time which may account for this increase in synthesizing ability. Also the increase in $PGF_2\alpha$ sythesizing ability on day -3 and -2 of the cycle in this group of animals suggests that the presence of an adjacent ovary is necessary for the normal manifestation of the $PGF_2\alpha$ sythesizing ability of the endometrium in the fistulated uterine horn.

EXPERIMENT V(B): Comparison of the effect of the presence and absence of an adjacent ovary on the content and sythesis of $PGF_2\alpha$ and PGE_2 by the uterus.

Objective:

In the present experiment a comparison has been made between uterine endometrial $PGF_2\alpha$ and PGE_2 content and synthesizing ability in a fistulated uterine horn in animals with either the ovary adjacent to the fistula present or absent. Thus the role of the ovary in prostaglandin production by the adjacent uterine horn is being investigated.

Animals and Methods

The animals used in this experiment form two groups:

Group 1

The 7 cycles from the 4 animals (ewes 50, 76, 86 and 88) cited in Exp. III which have a patent utero-mammary vein anastomosis and fistulation of uterine horn after the removal of the adjacent ovary. In addition, 2 cycles from a further animal (ewe 93) have been included in this group. This animal also underwent fistulation of the uterine horn after the removal of the adjacent ovary but in this case a patent anastomosis was not present. This animal's peripheral plasma levels have not been included in the calculations.

Group II

The 6 cycles from the 3 animals (ewe 67, 89 and 73) studied in part A of this experiment. The animals have an ovary remaining attached to the fistulated uterine horn. Simultaneous peripheral plasma samples were taken during only 4 of these cycles.

Thus daily samples of uterine endometrial tissue were taken during the latter part (day 12 onwards) of a total 15 oestrous cycles from 8 animals (9 cycles from 5 animals in group I; 6 cycles from 3 animals in group II). Simultaneous

venous blood samples were also taken during 11 of these cycles.

RESULTS

Figure 24 shows the pooled results obtained from sheep in group I and II. There was considerable variation in progesterone concentrations between individual animals resulting in the mean level for group I being higher than the mean for group II. However, this difference is mainly due to one animal (2 cycles of ewe 76) in group I, which had very high plasma progesterone concentrations. However, progesterone concentration in both groups fell normally at the end of the cycle indicating that luteal regression had taken place.

Plasma $PGF_2\alpha$ concentrations were higher in the animals which had a patent utero-mammary vein anastomosis (group I) than in those which lacked an anastomosis (group II). This would be expected since the uterine venous concentrations were measured in group I and peripheral concentrations in group II. One animal (ewe 93, fig 25) in group I did not have a patent anastomosis and as a result its peripheral $PGF_2\alpha$ levels were similar to those in group II. Thus these values have not been included in the group mean.

In the sheep in group I, there was no significant changes

Figure 24

Diagram of the means and standard errors of the means (S.E.M.) of the simultaneous concentration (ng mg^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$ (hatched bars) in the venous plasma, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the synthesizing ability (ng $mg^{-1}90$ min^{-1}) of endometrial tissue from these prostaglandins (open bars) during the latter part of the oestrous cycles in two groups of animals. Animals in group I have a patent utero-mammary vein anastomosis and fistulation of uterine horn after the removal of the adjacent ovary. However, animals in group II have no patent anastomosis and the ovary remaining attached to the fistulated uterine horn. The shaded bars below the abscissa indicates the days of oestrus.

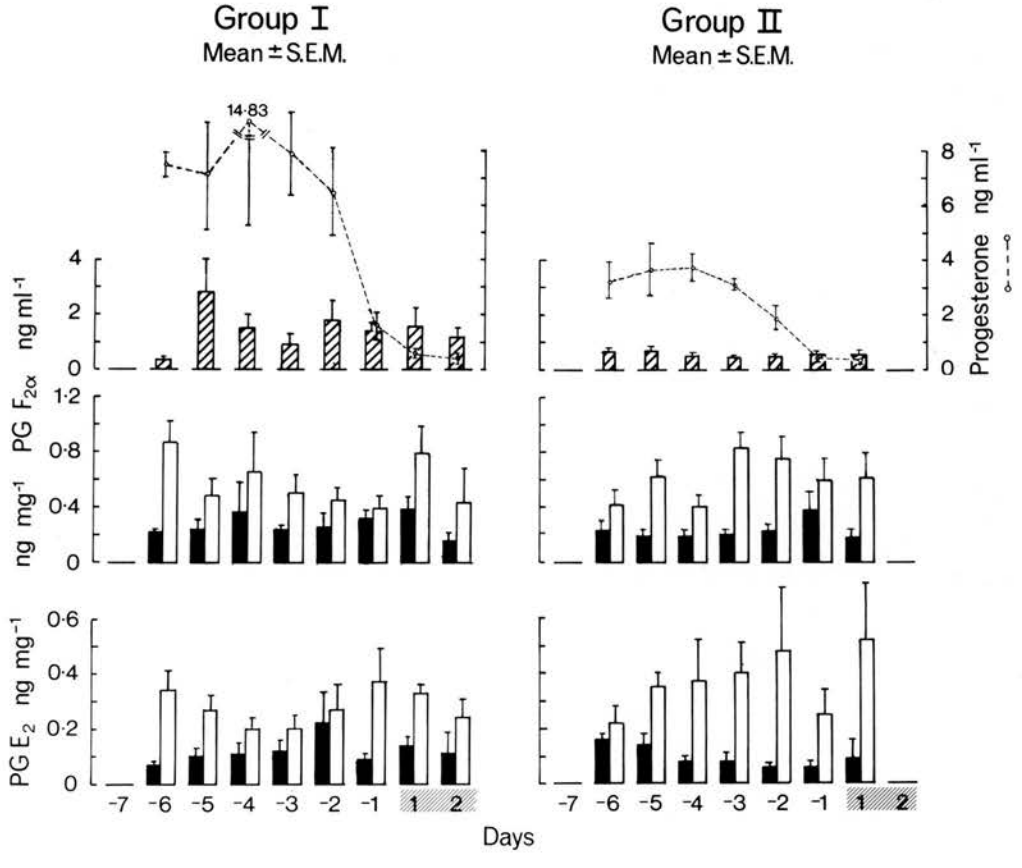
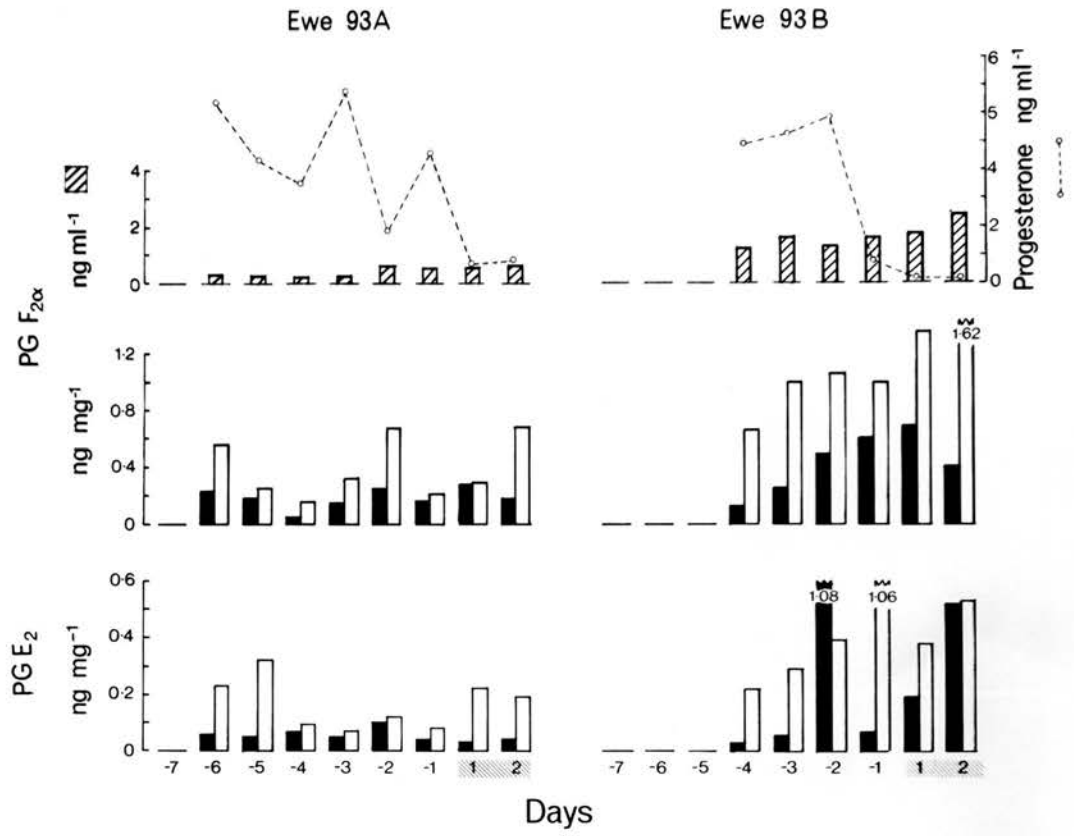

Group I
Mean ± S.E.M.
Group II
Mean ± S.E.M.
14·83
Progesterone ng ml⁻¹
PG F2α ng ml⁻¹
ng mg⁻¹
PG E2 ng mg⁻¹
-7 -6 -5 -4 -3 -2 -1 1 2
Days

Figure 25

Diagram of the simultaneous concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$ (hatched bars) in the mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PFE_2 (solid bars) and the synthesizing ability (ng mg^{-1} 90 min^{-1}) of endometrial tissue (open bars) during the latter part of two oestrous cycles (A and B) of ewe 93. In this ewe the ovary adjacent to the fistulated uterine horn had been removed. The shaded bars below the abscissa indicate the days of oestrus.


Ewe 93A
Ewe 93B
PG $F_{2\alpha}$
ng ml^{-1}
Progesterone ng ml^{-1}
ng mg^{-1}
PG E_2
1·62
1·08
1·06
Days

in endometrial $PGF_{2\alpha}$ and PGE_2 content but $PGF_{2\alpha}$ synthesizing ability was significantly higher on day 1 than that on day -2 ($p<0.01$) of the oestrous cycle. Also there was no correlation between endometrial $PGF_{2\alpha}$ content or synthesizing ability and the uterine venous plasma concentration of $PGF_{2\alpha}$ In the sheep in group II again there was no significant increase in endometrial $PGF_{2\alpha}$ content, but $PGF_{2\alpha}$ synthesizing ability again increased towards the end of the cycle but was significantly higher on days -3 ($p<0.02$) and -2 ($p<0.05$). Between group comparisons also showed that $PGF_{2\alpha}$ synthesizing ability was significantly higher on days -3 ($p<0.01$) and -2 ($p<0.05$) in group II. There was no significant differences in endometrial PGE_2 synthesizing ability within or between groups, consequently, there was no relationships between the ability of the endometrium to synthesize $PGF_{2\alpha}$ and PGE_2. As mentioned previously in both groups there was a direct relationship between endometrial content of $PGF_{2\alpha}$ and PGE_2.

DISCUSSION

No relationship existed between endometrial $PGF_{2\alpha}$ content, synthesizing ability and secretion in group 1 or content and synthesizing ability in group II animals. In group II a significant increase in the ability of uterus to synthesize $PGF_{2\alpha}$ occurred two and three days before the onset of oestrous cycle. This increase was not seen until day 1 of the oestrous cycle in group 1 animals. The

main difference between the animals in group I and II was the absence of an ovary adjacent to the fistulated uterine horn in the former group. Thus the increase in endometrial $PGF_2\alpha$ sythesizing ability two and three days before the onset of oestrus in animals where the adjacent ovary remain attached to the fistulated uterine horn (group II) but not until day 1 of the cycle when the ovary was removed (group I) suggests that the ovary exerts a local influence over endometrial $PGF_2\alpha$ sythesizing ability in the adjacent uterine horn.

EXPERIMENT VI: More frequent measurement of uterine $PGF_2\alpha$ and PGE_2 content, synthesis and secretion in the presence of an adjacent ovary.

Objective:

The results of the previous experiment (Exp. V A & B) showed that the presence of an adjacent ovary is necessary for the normal manifistation of the $PGF_2\alpha$ synthesizing ability of the endometrium in the fistulated uterine horn and suggest that the ovary exerts a local influence over endometrial $PGF_2\alpha$ synthesizing ability in the adjacent uterine horn.

Ideally in Exp. V, the simultaneous measurement of $PGF_2\alpha$ secretion into an anastomosed utero-mammary vein and its content and synthesizing ability in the endometrial tissue of the fistulated horn with an ovary attached to it, would have been preferable. But as mentioned previously (p.100) the weight of evidence on the pathway of the luteolytic mechanism implies that if such anastomosis is performed leaving the adjacent ovary in situ, this would lead to persistence of the corpus luteum. However, in their original experiments, Thorburn & Mattner (1971) did not find this even though they separated the uterine vein from the ovarian artery; an operation which should disrupt the counter-current mechanism whereby the uterine luteolysin could reach the ovary (see p.78 - 91). Furthermore, Lamond & Drost (1973) found that sectioning of the ovarian artery distal to the region where the counter-current transfer of $PGF_2\alpha$ is believed to take place did not interrupt the oestrous cycle. In addition, to these

experiments Coudert, Phillips, Faiman, Chernecki & Palmer (1974a, b) were unable to show transfer of radioactive xenon or Tritium-labelled $PGF_2\alpha$ from the uterine vein to the ovarian artery. All these experiments cast some doubt on the mechanism whereby the luteolysin would reach the ovary.

Thus in the current experiment preliminary attempts were made to anastomose the utero-ovarian vein from the fistulated uterine horn to the anterior mammary vein but leaving the adjacent ovary in situ in order to measure the secretion of $PGF_2\alpha$ from a fistulated uterine horn with an ovary remaining attached to it.

Animals and Methods

A single mature ewe (ewe 105) was used in this experiment. The animal was prepared and anaesthetized as described previously in Exp. I. The experimental procedure involved first the anastomosis of the utero-ovarian vein to the anterior mammary vein (as described in Exp. I) but leaving the ovary in situ, and then fistulation of the basal end of the same uterine horn to the exterior (as described in Exp. III). The fistulation was performed after the patency of the anastomosis had been checked by X-radiography (see Exp. I).

Samples were collected in the current experiment at various frequencies during two oestrous cycles. In the first cycle uterine venous blood samples from the anastomosed utero-mammary vein were

taken every three hours from day 13 to the onset of oestrus. However simultaneous endometrial biopsies were collected from the fistulated uterine horn every three hours only from day 13 to 16 of oestrous cycle and then daily biopsies (at 10.00 hr) were taken from day 17 to the onset of oestrus. In the second oestrous cycle, from day 16 to 18, uterine venous blood was taken from the anastomosed utero-mammary vein every half-hour and endometrial biopsies were taken every 8 hrs. The method used for collection and extraction of the samples are the same as those described in Exp. I & III.

RESULTS

Checking by X-radiography confirmed the patency of the anastomosis of the utero-ovarian vein to the mammary vein, thus as the blood samples were taken from anastomosed utero-mammary vein, the blood concentration of $PGF_2\alpha$ and progesterone taken in the two oestrous cycles studied are those of uterine venous blood. The ewe returned to oestrus in both cycles after a normal 18-day interval.

The concentrations of progesterone and $PGF_2\alpha$ in uterine venous plasma and the endometrial tissue content and synthesizing ability for $PGF_2\alpha$ and PGE_2 in the two cycle studies are illustrated individually in Figs 26 and 27.

As can be seen in Fig 26, the three hours samples taken during the first cycle showed that the concentrations of both progesterone and $PGF_2\alpha$ in the uterine venous blood fluctuated. $PGF_2\alpha$ show a series of peaks on days 13 and 14, this was later followed by an

Figure 26

Diagram of the concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$ (solid circles) in the anastomosed utero-mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the sythesizing ability (ng mg^{-1} 90 min^{-1}) of endometrial tissue (open bars) from the first cycle in ewe 105. The utero-mammary venous blood was collected every 3 hr from day 13 to the onset of oestrus while simultaneous 3 hr endometrial biopsies were taken only from day 13 to 16 of the oestrous cycle and then daily biopsies (at 10.00 hr) were taken from day 17 to the onset of oestrus. In this ewe the fistulated uterine horn had a patent utero-mammary vein anastomosis and its ovary was left in situ. The animal had a normal 18-day oestrous cycle.

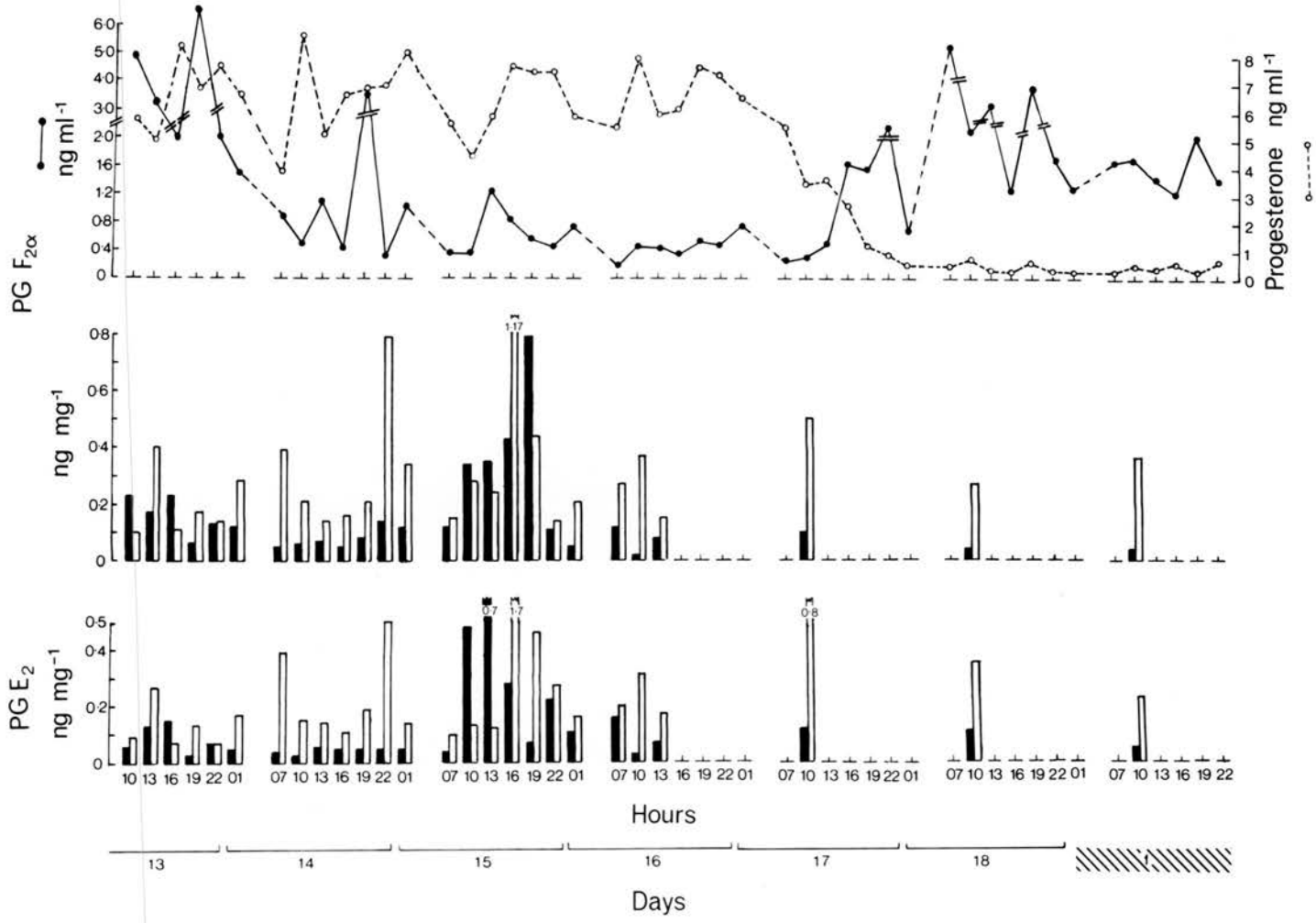
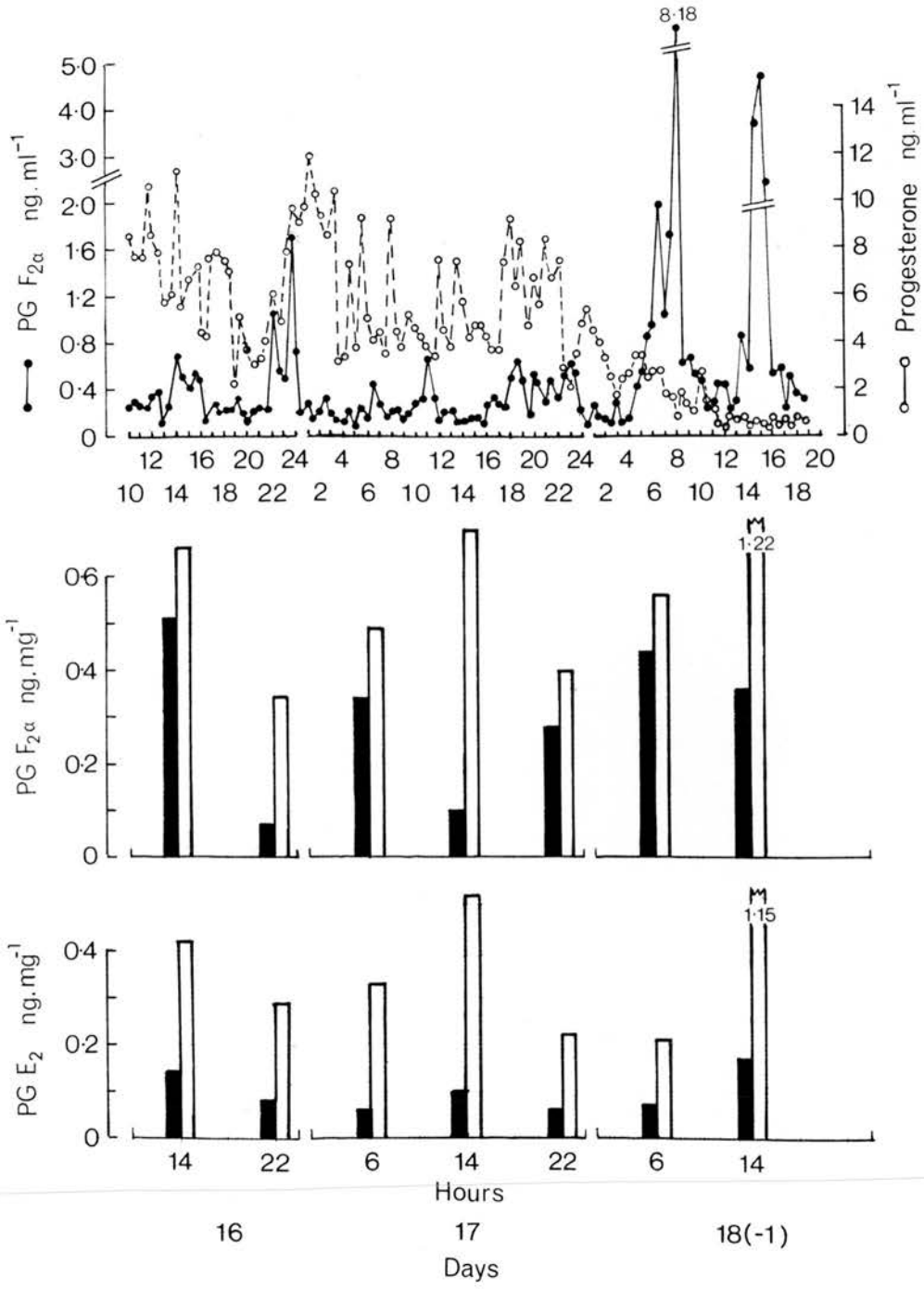

PG $F_{2\alpha}$
ng ml^{-1}
Progesterone ng ml^{-1}
ng mg^{-1}
PG E_2
ng mg^{-1}
1·17
0·7
1·7
0·8
10 13 16 19 22 01 07 10 13 16 19 22 01 07 10 13 16 19 22 01 07 10 13 16 19 22 01 07 10 13 16 19 22 01 07 10 13 16 19 22 01 07 10 13 16 19 22
Hours
13 14 15 16 17 18
Days

Figure 27

Diagram of concentration (ng ml^{-1}) of progesterone (open circles) and prostaglandin $F_2\alpha$(solid circles) in the anastomosed utero-mammary vein, the endometrial tissue content (ng mg^{-1}) of $PGF_2\alpha$ and PGE_2 (solid bars) and the synthesizing ability (ng mg^{-1} 90 min^{-1}) of endometrial tissue (open bars) from the second cycle in ewe 105. Throughout day 16, 17 and 18 of oestrous cycle, samples of utero-mammary venous blood were taken every half-hour and uterine endometrial biopsies were taken every 8 hr. In this ewe the fistulated uterine horn had a patent utero-mammary vein anastomosiss and its ovary was left in situ The animal had a normal 18-day cycle.

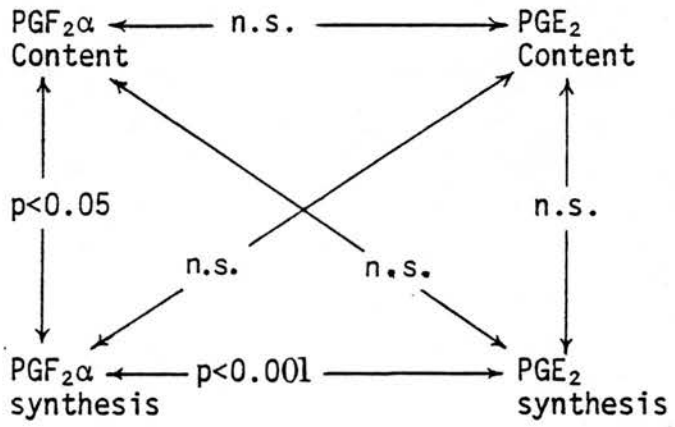

8·18
PG $F_{2\alpha}$ ng.ml^{-1}
Progesterone ng.ml^{-1}
5·0
4·0
3·0
2·0
1·6
1·2
0·8
0·4
0
14
12
10
8
6
4
2
0
12 16 20 24 4 8 12 16 20 24 4 8 12 16 20
10 14 18 22 2 6 10 14 18 22 2 6 10 14 18
1·22
PG $F_{2\alpha}$ ng.mg^{-1}
0·6
0·4
0·2
0
1·15
PG E_2 ng.mg^{-1}
0·4
0·2
0
14 22 6 14 22 6 14
Hours
16 17 18(-1)
Days

increase in $PGF_{2\alpha}$ concentration on day 17 to reach as high as 5.3 ng/ml on day 18. As this time progesterone concentrations were at the baseline level indicating that luteal regresson had already taken place (see Fig. 26). The episodic fluctuation of $PGF_{2\alpha}$ and progesterone are seen more clearly in the second cycle when half-hour samples were taken (see Fig. 27). A similar increase in $PGF_{2\alpha}$ concentration (to reach a maximum of 8.18 ng/ml) occurred on day 18 again at a time when luteolysis was almost complete and progesterone was at a low concentration (see Fig. 27). Moreover, a significant direct ($p<0.02$) relationship between the concentrations of progesterone and $PGF_{2\alpha}$ was demonstrated when the $PGF_{2\alpha}$ level was compared with progesterone level half-hour previously in the second cycle between 12.00 hr on day 16 and 4.00hronday 18. Also a significant ($p<0.01$) relationship was found between simultaneous plasma samples of $PGF_{2\alpha}$ and progesterone at the same period in the second cycle and even in the first cycle this relationship was approaching significance ($p\simeq0.08$), between 10.00 hr on day 13 and 13.00 hr on day 17. However, after 4.00 hr on day 18 in the second cycle and 13.00 hr on day 17 of the first cycle $PGF_{2\alpha}$ peaks were not related to progesterone secretion, since at this time progesterone levels were low due to CL regression.

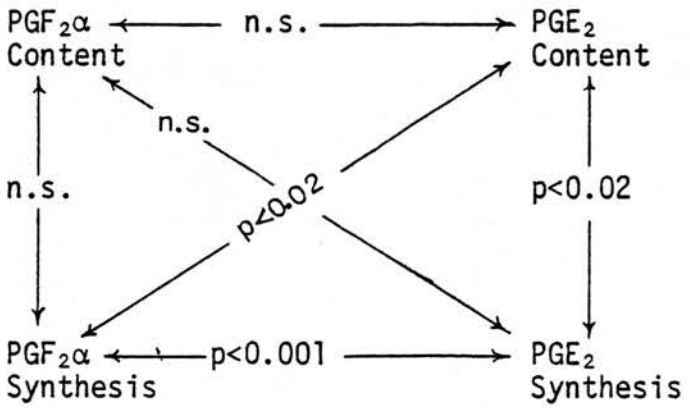
In both cycles statistical analysis of the results for relationships between simultaneous concentrations of $PGF_{2\alpha}$ in uterine venous plasma and endometrial tissue and between uterine plasma $PGF_{2\alpha}$ and endometrial synthesizi ability showed no significant relationships. Also no relationships were found between endometrial $PGF_{2\alpha}$ content or synthesizing ability and $PGF_{2\alpha}$ concentration in asynchronous plasma samples taken in either cycle. The significance of relationships between $PGF_{2\alpha}$ and PGE_2 content

and synthesizing ability in the uterine endometrial tissue of both cycles are shown below:-

1st Cycle



2nd Cycle



(n.s. = not significant)

DISCUSSION/

DISCUSSION

In the current experiments, the animals was found to have a normal 18-day oestrous cycle even when the ovarian artery was severed and the utero-ovarian vein anastomosed to the mammary vein, but leaving the ovary in situ. The fact that the ovary on the fistulated side was fully functional was confirmed by the presence of a corpus albicans in it at autopsy. Histological examination of this corpus albicans indicated that it had formed the sole functional corpus luteum during the second experimental cycle. Thorburn & Matter (1971) also reported normal oestrous cycles after separation of ovarian artery from the utero-ovarian vein in sheep and Lamond & Drost (1973) sectioned the ovarian artery distal to the region where the counter-current transfer of $PGF_2\alpha$ is believed to take place without interrupting the oestrous cycle. All these results cast some doubt on whether the uterine vein/ovarian artery counter-current mechanism is the only pathway involved in luteolysis.

The lower and less fluctuating level of progesterone in the first cycle is consistent with the histological finding that the luteal tissue during this cycle was in the ovary on the opposite side. The high concentration of $PGF_2\alpha$ at the end (day 18) of both oestrous cycles studied in the present experiment when progesterone is at its lowest level indicate

that high $PGF_{2\alpha}$ is required at the end of oestrous cycle to effect a complete luteolysis and prevent any functional recovery of the CL. A similar surge of $PGF_{2\alpha}$ at the end of luteal regression was reported by Thorburn, Cox, Currie Restall & Schnieder (1972, 1973) and in Exp. I & IV. A relationship was found between the simultaneous concentrations of progesterone and $PGF_{2\alpha}$ in the uterine venous blood in the two cycles. However, this relationship was even more demonstrable in the second cycle when the $PGF_{2\alpha}$ level was compared with progesterone level half-hour previously between 12.00 hr on day 16 and 4.00 hr on day 18, thus indicating that prior to luteal regression the increased $PGF_{2\alpha}$ release occurrred in response to an increase in progesterone secretion. A similar progesterone stimulation of $PGF_{2\alpha}$ release was found in ewe 76 in experiment IV.

The values of the content of $PGF_{2\alpha}$ in uterine caruncular and non-caruncular endometrial tissue found in this experiment are comparable to those found previously. During both cycles there was a highly significant ($p<0.001$) relationship between the endometrial ability to synthesize $PGF_{2\alpha}$ and PGE_2. A similar direct relationship was observed in cycle A of ewe 88 in Exp. IV (see p.118). This suggests that probably it is the availability of a common precursor that controls the synthesis of the two prostaglandins. No other significant relationships between the different parameters occurred in <u>both</u> cycles. Little can be concluded from the occurrence of apparently significant

relationships which only occurred in a single cycle, although it must be remembered that during the two cycles different parts of the cycle were examined in detail. The increase in both synthesizing ability (and content) of $PGF_2\alpha$ on day 15 (day -4) in the first cycle occurred only one day earlier than the similar increase in synthesizing ability found in animals with an ovary adjacent to the fistulated uterine horn in Exp. VA, even though the ovarian artery has been severed. The general absence of relationships between plasma concentration of $PGF_2\alpha$ and other parameters in the two cycles confirms the finding of the previous experiments and implies that the release of $PGF_2\alpha$ is under an independent control.

EXPERIMENT VII: Progesterone and prostaglandin $F_2\alpha$ concentration in various utero-ovarian vessels.

Objective:

As noticed in the previous experiment (Exp. VI) the mechanism by which $PGF_2\alpha$ reaches the ovary to cause the CL regression is still not fully clarified and it seems probable that another route is also involved in the transfer of this hormone. Thus in the present experiments the concentration of $PGF_2\alpha$ in the different uterine and ovarian vessels by which this hormone could be transferred have been measured. This may throw some light on other possible pathways of transfer. To complete the picture the concentration of progesterone was also measured in the different vessels and the presence or absence of the corpora lutea was noted.

Animals and Samples

Uterine and ovarian blood samples were collected from different vessels (i.e. uterine vein and artery, oviducal vein, ovarian vein and artery) from 28 mature non-pregnant Dorset Horn x Finnish Landrace ewes by laparotomy under anaesthesia on various days of 34 oestrous cycles. Peripheral blood samples were also collected from the mammary vein during most of these cyles as a control.

Anaesthesia was induced in all animals as previously described in Exp. II. During laparotomy the ovary was located and the presence or absence of corpora lutea noted. Blood samples (about 3 ml in each case) were than taken by simple venipuncture from different vessels. Each blood sample was collected in a sterile syringe containing one drop of Heparin (Pularin, Heparin injection. B.P., 12.500 $I.U.ml^{-1}$., Evans Medical Ltd., England) to prevent blood coagulation. Most ewes were sampled only once but if sampled a second time a suitable interval of at least 60-90 days was allowed to occur between samples. Ideally, the vessels adjacent to both ovaries were sampled however, this was not always possible because sampling was halted as soon as any signs of haematoma formation or oedema were observed as tissue damage itself can cause prostaglandin release (Greenwood & Kerry, 1975). In effect this meant that sampling had to be completed within 5-10 minutes. Blood samples were than centrifuged, stored and extracted as previously described in Exp. I. and $PGF_2\alpha$ and progesterone concentrations were measured.

RESULTS

The results of the present experiment are shown in Tables IV, V & VI. All the animals included in this experiment had a normal 16-19 days oestrous cycle. However, the length of oestrus varied from 1-2 days and the first day of oestrus was thus taken as day 1.

TABLE IV

The concentrations (ng ml^{-1}) of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and progesterone (prog.) in the mammary vein and in the various utero-ovarian vessels adjacent to an ovary bearing luteal tissue in sheep on different days of oestrous cycles.

Ewe No.	Day of Oestrous Cycle	Mammary Vein		Uterine Artery		Uterine Vein		Oviducal Vein		Ovarian Artery		Ovarian Vein	
		Prog. (ng ml^{-1})	$PGF_2\alpha$ (ng ml^{-1})	Prog. (ng ml^{-1})	$PGF_2\alpha$ (ng ml^{-1})	Prog. (ng ml^{-1})	$PGF_2\alpha$ (ng ml^{-1})	Prog. (ng ml^{-1})	$PGF_2\alpha$ (ng ml^{-1})	Prog. (ng ml^{-1})	$PGF_2\alpha$ (ng ml^{-1})	Prog. (ng ml^{-1})	$PGF_2\alpha$ (ng ml^{-1})
68	1			0.75	0.26	0.77	1.26			0.99	0.32	4.80	0.16
60	2			0.87	0.49	0.94	1.10	0.81	-	1.21	0.35	3.10	0.20
100	2			4.30	0.66	2.90	0.81	4.10	0.50				
103	2	7.20	0.53	4.90	0.65	6.90	3.40	15.8	1.20				
103	5	13.1	0.55 ★	9.00	0.6	12.6	0.50	9.30	1.35			22.6	0.90
				7.60	0.43	3.90	1.11	9.50	0.89				
109	8	4.70	0.13	5.20	0.04	4.50	0.22	12.8	0.32			910	0.19
68	11	4.50	0.17			5.04	0.18			4.50	0.19		
99	12	25.0	0.55 ★	13.7	0.84	13.7	0.48	15.3	0.87			35.8	1.30
				15.7	1.10	16.3	0.98	15.1	1.50			18.6	0.76
104	12		★	35.9	0.56	20.2	2.50	19.0	1.60			688	2.40
				27.3	0.30	3.90	2.10	27.2	3.20	31.4	0.51	61.5	0.45
87	12					15.3	1.56	13.3	0.64			277	1.03
105	12			65.5	0.66	73.0	0.80	67.0	3.20			343	1.03
101	12	46.6	0.84			121	9.40					53.3	1.30
98	13	16.5	0.80	41.3	1.10	12.7	0.77	27.0	1.10	15.5	1.00	920	1.10
108	13	6.60	0.36	5.40	0.47	6.70	1.59	9.80	0.25	34.3	0.36	195.7	0.30
109	13	14.3	0.68	12.8	0.66	13.9	5.60	15.3	3.70				
96	14	64.0	0.30	66.5	0.80	71.0	1.45	155.0	1.53			96.0	1.21
90	14			6.50	0.06	8.60	1.50	7.4	3.4				
57	14					3.64	2.42	9.89	0.65			14.94	0.72
85	14			9.90	0.62	5.30	5.20	5.1	3.50				
94	14			8.50	0.41	27.9	0.67	9.3	0.81	6.00	0.31	1070	0.15
78	14			8.80	0.26	23.4	3.22					1490	0.42
108	15	1.8	0.19	25.0	0.10	12.7	0.52	15.2	0.24	9.50	0.16	39.6	0.18
107	15	8.7	0.23	8.6	5.30	11.4	4.29	16.9	0.60				
65	15					27.0	0.50	18.6	0.29	56.0	0.29		
78	16					10.9	2.58	23.3	0.83				
104	16	14.1	0.20			12.4	4.50	13.3	0.65			263	0.19
96	16	6.80	0.69	4.80	2.80	44.6	5.80	38.1	4.60				
87	16			7.00	0.23	212	0.29					1360	0.07
99	16	70.5	0.70	73.0	0.48	81.0	1.02	69.0	-				
94	17			9.50	0.94	6.30	26.3	8.30	4.20	5.50	1.10	8.00	1.40

★ Opposite side of the same animal

TABLE V

The concentrations (ng ml^{-1}) of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and progesterone (Prog.) in the mammary vein and in the various utero-ovarian vessels adjacent to an ovary not containing a corpus luteum in sheep on different days of oestrous cycles.

Ewe No.	Day of Oestrous Cycle	Mammary Vein		Uterine Artery		Uterine Vein		Oviducal Vein		Ovarian Artery		Ovarian Vein	
		Prog. (ng ml^{-1})	$PGF_2\alpha$	Prog. (ng ml^{-1})	$PGF_2\alpha$	Prog. (ng ml^{-1})	$PGF_2\alpha$	Prog. (ng ml^{-1})	$PGF_2\alpha$	Prog. (ng ml^{-1})	$PGF_2\alpha$	Prog. (ng ml^{-1})	$PGF_2\alpha$
103	2	7.20	0.53	9.50	0.55	5.80	2.10	8.30	1.30			20.8	0.88
87	12					13.9	1.52	6.30	1.30				
108	13	6.60	0.36	7.50	8.50	6.80	8.75	6.10	8.55	8.4	0.46	12.9	1.33
96	14	64.0	0.30	17.5	0.67	80.0	4.60	20.0	0.20			77.0	0.77
57	14					4.22	1.10					4.67	1.33
108	15	1.80	0.19	5.90	0.74	11.7	1.14	18.2	0.22				
96	16	6.80	0.69	4.90	6.60	3.60	10.6	4.70	5.90			440	1.8
99	16	70.5	0.70	76.0	0.47	73.0	0.98	89.0	0.75				

TABLE VI

The concentration (ng ml^{-1}) of prostaglandin $F_2\alpha$($PGF_2\alpha$) and progesterone in the uterine venous blood draining the uterine horns adjacent to an ovary with (+CL) and without (-CL) luteal tissue in sheep on different days of oestrous cycle.

EWE NO.	Day of Oestrous Cycle	Progesterone (ng ml^{-1})		$PGF_2\alpha$ (ng ml^{-1})	
		+CL	-CL	+CL	-CL
103	2	6.90	5.80	3.40	2.10
77	4	16.2	10.6	0.44	0.36
74	8	73.2	7.40	0.41	0.28
87	12	15.3	13.9	1.51	1.53
101	12	121	45.6	9.41	9.10
108	13	6.70	6.80	1.59	8.75
109	13	13.9	11.9	5.60	8.00
57	14	3.64	4.22	2.42	1.10
96	14	71.0	80.0	1.45	4.60
95	14	8.80	8.10	0.74	0.77
97	14	8.30	14.1	4.50	3.50
94	14	27.9	61.1	0.67	1.43
65	15	27.0	9.40	0.5	0.75
108	15	12.7	11.7	0.52	1.14
87	16	212	39.0	0.29	0.76
96	16	44.6	3.60	5.80	10.6
99	16	81.0	73.0	1.02	0.98
94	17	6.30	6.80	26.3	27.2

As can be seen in Tables IV and V. There is no difference in the concentrations of either progesterone or $PGF_2\alpha$ in the uterine arterial plasma taken from the uterine horn adjacent to an ovary with or without corpus luteum and that of the mammary vein. Thus in the present results comparison has been made between the plasma concentrations of both hormones in various vessels with that of mammary vein and in some instances with uterine arterial plasma. The statistical analysis of comparison between these parameters are shown in table VII.

TABLE VII

The results of statistical analysis of $PGF_2\alpha$ and progesterone (Prog.) concentrations in the plasma of various vessels compared with that in peripheral plasma (when possible mammary vein plasma was taken as peripheral but in few instances uterine arterial plasma was used).

Status of the ovary	Uterine Vein ($ng\ ml^{-1}$)		Oviducal Vein ($ng\ ml^{-1}$)		Ovarian Vein ($ng\ ml^{-1}$)		Ovarian Artery ($ng\ ml^{-1}$)	
	Prog.	$PGF_2\alpha$	Prog.	$PGF_2\alpha$	Prog.	$PGF_2\alpha$	Prog.	$PGF_2\alpha$
with CL (Table IV)	n.s.	$p<0.01$	n.s.	$p<0.001$	$p<0.01$	$p<0.05$	n.s.	n.s.
without CL (Table V)	n.s.	n.s.	n.s.	n.s.	n.s.	$p<0.02$	-	-

n.s. = not significant
- = no comparison was made due to insufficient samples.

The concentration of $PGF_2\alpha$ and progesterone in the uterine venous plasma taken from the uterine vein adjacent to an ovary with or without CL are shown in Table VI. Although overall no significant differences have been found between the concentrations of the hormones in the two sides, plasma progesterone concentration in the uterine vein adjacent to an ovary bearing the CL was higher than that of the vein adjacent to an ovary without CL on all days of oestrous cycles except day 14.

DISCUSSION

The results of Exp. VI suggest that beside the counter-current transfer mechanism whereby $PGF_2\alpha$ could pass from the uterine vein into the ovarian artery, another route is possibly involved in the transfer of sufficient amount of this hormone to the ovary. In the current experiment, concentration of $PGF_2\alpha$ significantly higher than peripheral levels have been found in the uterine vein ($p<0.01$), the oviducal vein ($p<0.001$) and the ovarian vein ($p<0.05$) on all days of oestrous cycle. This suggests that the alternative route whereby $PGF_2\alpha$ can reach the ovary is via the venous drainage passing alongside the oviduct. This vein besides itself being involved in the vascular plexus in ovarian pedicle also anastomoses with the ovarian vein which is a major constituent of this plexus (Del Campo & Ginther, 1973b). McCracken & Einer-Jensen (1976) suggested that the vascular plexus in the ovarian pedicle may form a counter-current system whereby ovarian steroids could feedback and regulate further ovarian steroid production. Such a feedback

has been found by Walsh, Yutrzenka & Davis (1979), although Caffrey, Nett, Abel & Niswender (1979) were unable to confirm its existence. The current data suggest that this vascular plexus in the ovarian pedicle may be involved in the transfer of $PGF_{2\alpha}$ from the oviducal and ovarian veins into the ovarian artery. It is interesting to note that Baird & Land (1973) found that only 4 out of 10 ewes, in which the main uterine vein alone was ligated and divided showed perisistent luteal function. In the ewes which returned to heat, the tubal branches of the uterine vein had formed anastomoses between the uterine and ovarian veins. Division of these anastomotic veins in addition to main uterine vein inhibited luteal regression in 6 out of 7 ewes. The presence of these tubal venous arcades of anastomosing vessels also can explain the similar results obtained by other workers (e.g. Kiracofe et al. 1966; Lamond & Drost, 1973). However, the utero-ovarian vascular system in sheep is dynamic and readily compensates for intervention and must be interpreted with great care. For instance, Ginther (1974) suggested that the relative amount of uterine venous effluent which discharged through the oviducal veins must be small in normal ewes, but could be considerable after ligation of the main uterine vein. We found that even in normal animals the normal importance of the oviducal veins may vary, for the oviducal veins in some of the animals operated were large and prominent while in others they were very small.

Table VII appears to show that more $PGF_{2\alpha}$ was released from the

uterus on the side containing the CL, however this is not substantiated by the data from the uterine vein shown in Table VI, and so must be treated with caution.

In conclusion the results of the current experiment suggest that $PGF_2\alpha$ may reach the ovary by the following two routes:

1. Via the uterine vein and then by transfer from the utero-ovarian vein to the ovarian artery by counter-current mechanism as previously described (see p. 78).

2. Via the oviducal vein and its anastomoses with the ovarian vein and then by transfer from the ovarian vein to the ovarian artery in the ovarian pedicle.

EXPERIMENT VIII: Effect of long-term progesterone injection on uterine production and release of prostaglandins.

Objective:

Incidental to the collection of uterine fluid for evaluating the 6-oxo-$PGF_1\alpha$assay, a small investigation into the effect of progesterone injections on uterine prostaglandin production and release was undertaken.

Animals and Methods

Two non-pregnant Dorset Horn x Finnish Landrace ewes were used. Both animals (ewe 82 and 83) were injected intramuscularly daily with 10 mg of progesterone either in 1 or 2 ml of Olive or Arachis oil. Laparotomy was performed at 94 and 155 days after the start of progesterone treatment in ewe 82 and at 96, 154 and 201 days in ewe 83. Fluid present in the uterus at laparotomy was aspirated with a sterile syringe, a small sample was taken for bacteriological examination and the remainder stored at -20°C. Samples of uterine venous and arterial, and anterior mammary vein, blood were taken at the time of laparotomy. The blood was centrifuged and plasma stored at -20°C. Duplicate biopsies of caruncular and non-caruncular endometrium were also obtained. At both laparotomy and postmortem, samples of endometrium were taken for histology. $PGF_2\alpha$ and progesterone in the uterine fluid and plasma samples were extracted as described previously in Exp. I and the endometrial samples were extracted as those in Exp. II.

RESULTS

Both ewes were checked daily with a vasectomized ram but neither displayed oestrus while being injected with progesterone . At laparotomy on all occasions the uterus was distended by the fluid and the ovaries contained no corpora lutea or large follicles. Fluid withdrawn from the uterus was sterile, odourless and viscous and was present in amounts that ranged from 30 to 450 ml (see Table VIII). In all five cases prostaglandin $F_2\alpha$ was found in the uterine fluid and ranged in concentration from 8.4 to 450 ng ml^{-1}. The highest concentration of $PGF_2\alpha$ was found in ewe 82 on day 155. This sheep also yielded the largest amount of uterine fluid. By comparison, only small quantities of PGE_2 (ranging from 0.18 to 6.3 ng ml^{-1})were found in the uterine fluid (see Table VIII). Progesterone was also detected in the uterine fluid in all cases in a concentration ranged from 0.28 to 3.26 ng ml^{-1}.

TABLE VIII The amount of uterine fluid and the concentration of some of its components (progesterone, $PGF_2\alpha$ and PGE_2) at various times during progesterone injections in sheep.

Ewe No.	Days after last oestrus	Amount of uterine fluid	Progesterone (ng ml^{-1})	$PGF_2\alpha$ (ng ml^{-1})	PGE_2 (ng ml^{-1})
82	94	200	1.10	256.0	2.5
	155	450	1.90	450.0	3.3
83	96	30	0.28	8.40	0.18
	154	40	3.26	90.30	4.16
	201	30	2.70	89.10	6.3

In addition, the uterine venous blood contained large amounts of $PGF_2\alpha$(up to 49.6 ng ml^{-1}),while lower concentrations were found in uterine artery and in the peripheral blood (see Table IX). Naturally high concentration of progesterone was found in both the uterine venous and arterial blood .

TABLE IX Plasma concentrations of progesterone (Prog.) and $PGF_2\alpha$ in various blood vessels at various times during progesterone injections in sheep (compared with Table VIII).

Ewe No.	Days after last oestrus	Uterine Vein (ng ml^{-1})		Uterine artery (ng ml^{-1})		Mammary vein (ng ml^{-1})	
		Prog.	$PGF_2\alpha$	Prog.	$PGF_2\alpha$	Prog.	$PGF_2\alpha$
82	94	13.0	10.4	-	-	-	-
	155	274.6	47.2	12.9	14.0	15.0	0.73
83	96	12.5	2.63	13.9	0.34	8.7	0.22
	154	20.25	2.0	25.5	2.60	-	-
	201	6.3	49.6	7.5	0.57	25.2	2.28

Biopsy specimens of caruncular and non-caruncular endometrial tissue were removed from the uterus at laparotomy and at autopsy. Histologically the uterine endometrium was very thin and both the luminal and glandular epithelium was found to be comprised of tall columnar cells similar to those found in the luteal phase of the normal oestrous cycles (Harrison, Heap, Horton & Poyser, 1972). The $PGF_2\alpha$ and PGE_2

content and synthesizing ability of caruncular and non-caruncular endometrial tissue showed no consistent trends (see Table X), but was comparable to and not significantly different from those in the late luteal phase of the normal oestrous cycle.

TABLE X : Prostaglandin $F_{2\alpha}$ and PGE_2 content and synthesizing ability of uterine tissue at various times during progesterone injections in sheep (compared with Tables VIII & IX).

Ewe No.	Days after last oestrus	Type of Tissue	Content (ng mg^{-1})		Synthesizing ability (ng mg^{-1} 90 min^{-1})	
			$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$	PGE_2
82	94	Caruncle	0.41	0.016	2.03	0.48
	155	Caruncle	0.20	0.015	0.31	0.98
		Endometrium	0.31	0.008	0.11	0.02
83	96	Caruncle	0.71	0.005	0.28	0.24
	154	Caruncle	0.74	0.613	0.78	0.23
	201	Caruncle	0.28	0.036	0.69	0.21
		Endometrium	0.70	0.104	0.49	0.07

DISCUSSION

It is well known that there is little fluid in the uterine lumen at any time during the normal oestrous cycle in sheep. No fluids was shown to accumulate in the uterus when both the uterus and ovary were transplanted together and the animal showed normal cyclical behaviour (Harrison, Heap & Linzell, 1968). However,

large quantities of uterine fluid accumulate after transplantation of either the uterus (Goding, McCracken & Baird, 1967b) or the ovary seperately (Harrison, Heap, Horton & Poyser, 1972) and the transplantation in both cases led to persistent luteal function. Moreover, the daily injection of progesterone to a ewe from which one ovary was removed led to accumulation of fluid in the uterus (Amoroso, Harrison, Heap & Poyser, 1973; Harrison, Heap & Poyser, 1976). This fluid was found to be rich in $PGF_2\alpha$.

In the current experiments the accumulated fluid was sterile, viscous and odourless as was that reported by Harrison et al. (1972). and contained a high concentration of $PGF_2\alpha$ in comparison with PGE_2. Prostaglandin $F_2\alpha$ and E_2 was also detected in the uterine endometrial tissue samples. If breakdown of the endometrium had been the cause of the presence of $PGF_2\alpha$ in the uterine fluid, appreciable quantities of PGE_2 would also be expected to be present in the uterine fluid. However, the relatively low concentration of PGE_2 in the uterine fluid must indicate a high secretion rate of $PGF_2\alpha$ by the uterus. Thus the high concentration of circulating progesterone has led to an accumulation of the $PGF_2\alpha$ - rich fluid in the uterus. As previously described by Amoroso et al. (1973) and Harrison et al. (1976), $PGF_2\alpha$ was also present in higher amounts in the uterine venous blood than in the uterine arterial and peripheral blood, thus it appears that the uterus was also secreting a large amount of $PGF_2\alpha$ into the circulation as well as into the uterine lumen. This increased secretion of $PGF_2\alpha$ in response to high progesterone agrees with

the results of Exp. VII and the half-hour samples in Exp. IV and VI, that increase in progesterone secretion by the CL led to increase in $PGF_2\alpha$ release. The content of $PGF_2\alpha$ and PGE_2 in the endometrial tissue and the ability of these tissues to synthesize the two prostaglandins was found to be similar to that at the luteal phase of the normal oestrous cycle.

GENERAL DISCUSSION

Experiments II, III, IV, V and VI demonstrated that the values for the content of $PGF_{2\alpha}$ in the uterine caruncular and non-caruncular endometrial tissue are comparable to those found by previous workers (Wilson, Cenedella, Butcher & Inskeep, 1972; Lewis et al. 1977 & 1978). Also no difference was found between $PGF_{2\alpha}$ and PGE_2 content and synthesizing ability of the two types of uterine tissue. However, in Exp. II, we, like Pexton et al.(1975), have found significantly lower concentration of $PGF_{2\alpha}$ in the myometrium. This would explain the apparently contradictory findings reported by Louis, Parry, Robinson, Thorburn & Challis (1977), that the intercaruncular tissue which consisted of endometrium and myometrium contained less and produce less $PGF_{2\alpha}$ than did the caruncular tissue. Thus, it appears that this difference was probaly due to the inclusion of myometrium in their inter-caruncular tissue.

The results of Exp. II demonstrated a significantly higher $PGF_{2\alpha}$ content in the second half of the oestrous cycle. Also in Exp. VA there was a significant increase of $PGF_{2\alpha}$ synthesizing ability of uterus 3 days before oestrus which coincided with the commencement of the fall in plasma progesterone. A similar increase in the ability of uterine tissue to synthesize prostaglandins at the end of the cycle has been reported in the guinea-pig (Poyser, 1972). Furthermore in this latter species the increase in the uterine prostaglandin production and release mainly involved $PGF_{2\alpha}$ (Poyser, 1973; 1974 a,b). The increase in production has been confirmed by Wlodawer, Kindahl & Hamberg (1976) using microsomal preparations of guinea-pig uterus and exogenous arachidonic acid.

The very high plasma concentration of $PGF_2\alpha$ at the end of oestrous cycle (Exp. I, IV and VI) when progesterone levels have already fallen suggest that a major role of $PGF_2\alpha$ at the end of oestrous cycle is to complete luteolysis and to prevent any functional recovery of the CL. Similarly high concentrations of $PGF_2\alpha$ at this time were reported by Thorburn, Cox, Currie, Restall & Schneider (1972, 1973). The significant relationships between the concentration of progesterone and $PGF_2\alpha$ in the uterine venous blood in ewe 76 (Exp. IV) and in the second cycle of ewe 105 (Exp. VI) was most demonstrable when the $PGF_2\alpha$ level was compared with the progesterone level half-hour previously. This indicats that prior to the cessation of progesterone secretion the increased $PGF_2\alpha$ release may occur in response to episodic increase in progesterone output, especially as stated in introduction that progesterone alone will induce $PGF_2\alpha$ secretion from the sheep uterus. However, the effect of immunizing sheep against progesterone (Fairclough, Smith, Peterson & McGowan, 1976) does not fit with this hypothesis. Fairclough and co-workers (1976) found normal length oestrous cycles after immunizing sheep against progesterone. This apparent absence of an effect after removing progesterone may be due to the antisera only removing just over half (50 - 75%) of the free progesterone from the circulation. This residual amount of progesterone may have been enough to maintain a normal cycle.

The absence of any significant changes in either the content and synthesizing ability of 6-oxo-$PGF_1\alpha$ of the different uterine tissues (Exp. 11) indicates that this compound, like PGE_2, is probably not involved in luteolysis.

The relationships between simultaneous measurement of uterine content of $PGF_{2\alpha}$ and PGE_2, the ability of the endometrial tissue to synthesize prostaglandins and the concentration of $PGF_{2\alpha}$ in the uterine venous blood (Exp. III, IV, VA, B and VI) should indicate the area of prostaglandin synthesis at which extra-uterine factors could operate to cause the increase in the release of $PGF_{2\alpha}$ needed for luteolysis. The highly significant relationships ($p<0.001$) between the ability of endometrial tissue to synthesize $PGF_{2\alpha}$ and PGE_2 found in Exp. IV (cycle A of ewe 88) and Exp. VI (both cycles) and the direct relationships between the endometrial content of $PGF_{2\alpha}$ and PGE_2 found in Exp. III, VA & B suggest either that PGE_2 production is a by-product of $PGF_{2\alpha}$ production or that it is the availability of a common precursor that controls the synthesis of the two prostaglandins. The latter is improbable as the addition of 20 μg arachidonic acid to one sample in paired incubates (Exp. III & IV) failed to significantly alter the amount of $PGF_{2\alpha}$ or PGE_2 synthesized. This indeed suggests that the availability of this precursor was not the limiting factor in the synthesis of $PGF_{2\alpha}$ and PGE_2, at least in this incubation system. Likewise in the guinea-pig it has been shown that the smaller amounts of $PGF_{2\alpha}$ produced by uterine homogenates during the early part of the cycle were not due to the lack of precursor in the incubate, as addition of exogenous arachidonic acid (2.5 μg ml^{-1}) to day 7 uterine homogenates did not increase the yield of $PGF_{2\alpha}$ following the incubation (Poyser, 1971). Also the difference in the synthesizing capacity of the guinea-pig uterus <u>in vitro</u> between days early and late in the cycle is not due to difference in exogenous substrate level or difference in metabolism (Maule Walker in Poyser, 1976; Mitchell, Poyser & Wilson, 1977; Poyser, 1978 a), but probably it reflects an increase in the level of $PGF_{2\alpha}$ synthesized due to an increase in the amount of prostaglandin synthetase within the uterus. The involvement of

enzyme levels was investigated by studying the effect of actinomycin D, a protein synthesis inhibitor, on the prostaglandin synthesizing capacity of the uterus and oestrous cycle length in guinea-pig (Poyser, 1979). Actinomycin D apparently prevented the increase in the uterine prostaglandin synthetase level that normally takes place after day 11, thereby reducing uterine $PGF_{2\alpha}$ synthesis and output in vivo and resulting in luteal maintenance and longer oestrous cycle. Thus in the guinea-pig, the increase in the ability of the uterus to synthesize $PGF_{2\alpha}$ in the latter part of the oestrous cycle appears to be due to an increase in uterine prostaglandin synthetase (Poyser, 1978 a). It is very significant that Smith, Husling & Fogwell (1979) have recently reported that in the sheep there is an increase in prostaglandin-forming cyclo-oxygenase which coincides with the increase in the synthesizing ability of the uterus for $PGF_{2\alpha}$ found in the present thesis. Thus the increased release of $PGF_{2\alpha}$ in the latter part of the cycle seems to depend on an increase in the efficiency with which arachidonate is converted to $PGF_{2\alpha}$-precursor rather than on the availability of the substrate. Moreover, similar to that in guinea-pig, French & Casida (1973) reported that intrauterine administration of actinomycin D prevents luteal regression in sheep, which may indicate that uterine prostaglandin synthetase levels are again involved in controlling $PGF_{2\alpha}$ synthesis and output.

Little can be concluded from the occurrence of apparent significant relationships which only occurred on a few occasions, i.e. the content of $PGF_{2\alpha}$ and its synthesizing ability in Exp. IV (cycle B of ewe 88) and Exp. IV (first cycle); the content of PGE_2 and the synthesizing ability of $PGF_{2\alpha}$ in the second cycle of Exp. VI; and the plasma concentration of $PGF_{2\alpha}$ and its endometrial content in ewe 76 of Exp. IV. The general absence of relationships between plasma concentrations of $PGF_{2\alpha}$ and its endometrial content and synthesizing ability in Exps. IV, VI & VII is surprising and implies that the release of $PGF_{2\alpha}$ is under independent control. However,

a significant relationship was found between simultaneous plasma samples of $PGF_{2\alpha}$ and progesterone in the second cycle of ewe 105 (Exp. VI) and even in the first cycle in this animal the relationship was approaching significance ($p \simeq 0.08$). It should be noted that this animal is the only one in which plasma samples from the uterine vein were taken with an adjacent ovary present. The absence of the relationships between simultaneous measurement of progesterone and $PGF_{2\alpha}$ in all the other animals may be due to the absence of the ovary especially in the light of the findings in Exp. V. Further work is required to clarify this point.

The effect of the absence or presence of the ovary adjacent to the fistulated uterine horn produced some interesting results: It was found that the endometrial synthesizing ability of $PGF_{2\alpha}$ was significantly increased four (in the first cycle of Exp. VI), three and two days (in Exp. VA and group II in Exp. VB) before the onset of oestrus in sheep with an ovary adjacent to the fistulated uterine horn, but this increase was delayed until day 1 in ewes with the adjacent ovary removed. Likewise the between groups comparisons in Exp. VB showed that $PGF_{2\alpha}$ synthesizing ability was only significantly higher at three and two days before oestrus in the group with an ovary adjacent to the fistulated uterine horn but not at any time in the other group. These results indicate that the presence of an ovary adjacent to the uterine horn is necessary for the normal manifestation of increased $PGF_{2\alpha}$ synthesizing ability of the endometrium in the fistulated uterine horn and suggest that the ovary exerts a local influence over endometrial $PGF_{2\alpha}$ synthesizing ability in the adjacent uterine tissue.

Experiment VI showed that the anastomosis of the utero-ovarian vein from the fistulated uterine horn, to the anterior mammary vein but leaving the adjacent ovary <u>in situ</u> did not interrupt the normal oestrous cycle as was previously anticipated. This finding suggests that another route is <u>also</u> involved in the transfer of $PGF_{2\alpha}$ to the adjacent ovary in addition

to its normal way through the counter-current mechanism of transfer by diffusing from the uterine vein to the ovarian artery. The results of this experiment are consistent with those reported by Baird & Land (1973) (see p.18). In Exp. VII it was found that the concentration of $PGF_{2\alpha}$ in the oviducal vein and the ovarian vein, as well as in the uterine vein were significantly higher than peripheral levels. This indicates that the alternative route is probably via the oviducal vein and its anastomoses with the ovarian vein and then by diffusion from the ovarian vein to the ovarian artery in the ovarian pedicle. This would also give a second function to the vascular plexus in the ovarian pedicle besides the local feedback of ovarian progesterone described by Walsh, Yu trzenka & Davis (1979) and also confirmsthe local extra-ovarian pathway proposed by Fogwell, Lewis , Butcher & Inskeep (1977) to explain the occurrence of luteolysis following injection of $PGF_{2\alpha}$ into an ovarian follicle (p.79).

The last experiment (Exp. VIII) confirmed the finding of Amoroso, Harrison, Heap & Poyser (1973) and Harrison, Heap & Poyser (1976) and showed that daily injection of progesterone to sheep led to accummulation of large amounts of fluid in the uterus. This uterine fluid was found to be rich in $PGF_{2\alpha}$ but only very small amounts of PGE_2 were present. Concentrations of $PGF_{2\alpha}$ higher than those in either uterine arterial or peripheral blood were also found in the uterine venous blood. Thus, it appears that the uterus was secreting a large amount of $PGF_{2\alpha}$ into the circulation as well as into the uterine lumen even although the endometrial content of $PGF_{2\alpha}$ and PGE_2 and the ability of this tissue to synthesize both prostaglandins was found only to be similar to that during the luteal phase of normal oestrouscycle. These findings suggest that the high concentration of progesterone is causing $PGF_{2\alpha}$ release and is reminiscent of the effect of the corpus luteum in Exp. VII. However, in view of the absence of oestrogen

measurements in these experiments and the weight of evidence (see p.98) in favour of oestrogen causing $PGF_{2\alpha}$ release it would be premature to suggest that it is progesterone alone that caused $PGF_{2\alpha}$ release at the end of the oestrous cycle. The conclusion from previous work (p.82) was that oestrogen was important in the actual release of $PGF_{2\alpha}$ while progesterone was probably necessary for $PGF_{2\alpha}$ synthesis. However, the work of Fairclough, Smith, Peterson & McGowan (1976) appears to contradict the role of oestradial in $PGF_{2\alpha}$ synthesis. These workers found that the immunization of sheep against oestradiol did not interfere with luteolysis. But as the injection of antisera was not commenced until day 9 and plasma oestradial did not decrease until day 13, it is possible that this was too late to interfere with oestradiol involved in luteolysis. The increase in $PGF_{2\alpha}$ synthesis alone on days -3 and -2 in Exp. VA, when luteal tissue must have been present on the fistulated side in 5 of 6 cycles, would support the involvement of progesterone in the increase in $PGF_{2\alpha}$ synthesizing capacity. It is clear from Exp. VII and VIII, and the half-hour samples in Exp. IV and VI that progesterone is also involved in $PGF_{2\alpha}$ release.

In the rat and guinea-pig progesterone seems to play an even lesser role in $PGF_{2\alpha}$ synthesis than in the sheep. For example, in the ovarictomized guinea-pig althrough progesterone pretreatment followed by oestradiol resulted in a higher concentration of $PGF_{2\alpha}$ in the utero-ovarian venous blood than when either hormone was given alone (Blatchley & Poyser, 1974), only oestradiol treatment increased the synthesizing capacity of the uterus (Naylor & Poyser, 1975). Progesterone had no effect nor did it modify the response of the uterus to oestradiol. Furthermore, in the rat oestradiol has been shown to increase the levels of uterine prostaglandin synthetase (Ham, Cirillo, Zenetti & Kuehl, 1975).

From all these data it seems that in guinea-pig, rat and possibly in sheep, it is the increase in prostaglandin synthetase that increases the $PGF_{2\alpha}$ production and this seems to be under the control of oestrogen

working on a progesterone-primed uterus especially in the sheep, since oestrogen concentrations rise and progesterone falls at the right time. Further work is required to clarify whether oestrogen (with progesterone) is the only stimulus involved in this mechanism, especially in view of the work suggesting that uterine release of $PGF_{2}\alpha$ may be controlled by oxytocin, and that oestradiol potentiates oxytocin-induced $PGF_{2}\alpha$ release by inducing an increase in the number of endometrial oxytocin receptors (see p. 91 - 92).

If oestradiol released from one ovary could selectively increase prostaglandin synthetase level in its adjacent uterine horn, this would account for the unilateral effect reported in this thesis. Immunization of sheep against oestradiol starting earlier in the cycle than Fairclough et al. (1976) would help to clarify this situation.

In addition full exploitation of the experimental preparation used in Exp. VI, in which the ovary was retained adjacent to the fistulated uterine horn, and frequent monitoring of oestradiol progesterone and $PGF_{2}\alpha$ would help in solving this complex problem.

Obviously, a lot more evidence is needed to clarify the relative importance of oestrogen, progesterone and oxytocin in $PGF_{2}\alpha$ synthesis and release from the endometrium in vivo in sheep.

REFERENCES

Acritopoulou, S., Haresign, W. & Lamming, G.E. (1978). Time of ovulation in ewes after treatment with a prostaglandin $F_{2\alpha}$ analogue. Journal of Reproduction & Fertility 54, 189-191.

Adams, J.H., Daniel, P.M. & Pri c hard, M.K.L. (1963). Distribution of hypophysial portal blood in anterior lobe of the pituitary gland. Endocrinology 75, 120-126.

Akbar, A.M., Rowe, K.E. & Stormshak, F. (1970). Luteal regression by estradiol in unilaterally hysterectomized ewes. Journal of Animal Science 30, 1033.

Akbar, A.M., Rowe, K.E. & Stormshak, F. (1971). Estradiol induced luteal regression in unilaterally hysterectomized and luteinizing hormone-treated ewes. Journal of Animal Science 33, 426-429.

Aldridge, R.R., Barrett, S. Brown, J.B., Funder, J.W., Goding, J.R., Kaltenbach, C.C. & Mole, B.J. (1970). The effect of prostaglandins on ovarian steroidogenesis in vivo. Journal of Reproduction & Fertility 21, 369-370.

Amoroso, E.C., Harrison, F.A., Heap, P.B. & Poyser, N.L. (1973). The production of prostaglandin $F_{2\alpha}$ by the uterus of the sheep. Journal of Endocrinology 57, lix.

Anderson, L.L., Bland, K.P. & Melampy, R.M. (1969). Comparative aspects of uterine luteal relationships. Recent progress in Hormone Research 25, 57-104.

Anderson, L.L., Bowerman, A.M. & Melampy, R.M. (1963). Neuro-utero-ovarian relationships. In: Advances in Neuroendocrinology, A.V. Nalbandov, ed., p 345-373, University of Illinois Press, Urbana.

Armstrong, D.T. (1968). In vitro synthesis of progesterone. Journal of Animal Science 27, Suppl. 1, 181-203.

Armstrong, D.T. & Hansel, W. (1959). Alteration of the bovine estrous cycle with oxytocin. Journal of Dairy Science 42, 533.

Baird, D.T. (1974). Prostaglandin $F_{2\alpha}$ and ovarian blood flow in sheep. Journal of Endocrinology 62, 413-414.

Baird, D.T., Collett, R.A. & Land, R.B. (1971). The pattern of progesterone secretion by the autotransplanted ovary of the ewe in response to ovine luteinizing hormone and HCG. Journal of Reproduction & Fertility 25, 308-309.

Baird, D.T., Goding, J.R., Ichikawa, Y. & McCracken, J.A. (1968). The secretion of steroids from the autotransplanted ovary in the ewe; spontaneously and in response to systemic gonadotrophin. Journal of Endocrinology 42, 283-299.

Baird, D.T., & Land, R.B. (1973). Division of the uterine vein and the function of the adjacent ovary in the ewe. Journal of Reproduction & Fertility 33, 393-397.

Baird, D.T., Land, R.B., Scaramuzzi, R.J. & Wheeler, A.G. (1976). Endocrine changes associated with luteal regression in the ewe; the secretion of ovarian oestradiol, progesterone and androstenedione and uterine prostaglandin $F_{2\alpha}$ throughout the oestrous cycle. Journal of Endocrinology 69, 275-286.

Baird, D.T., McCracken, J.A. & Goding, J.R. (1973). Studies in steroid synthesis and secretion with the autotransplanted sheep ovary and adrenal. In: The Endocrinology of Pregnancy and Partuirition, Experimental Studies in Sheep, P 5-16. Ed. C.G. Pierrepoint. Alpha Omega Alpha Publishing, Cardiff, Wales.

Baird, D.T. & Scaramuzzi, R.J. (1976). Changes in the secretion of ovarian steroids and pituitary luteinizing hormone in the peri-ovulatory period in the ewe: The effect of progesterone. Journal of Endocrinology 70, 237-245.

Barcikowski, B., Carlson, J.C.,Wilson, L. & McCracken, J.A. (1974). The effect of endogenous and exogenous estradiol-17β on the release of prostaglandin $F_{2\alpha}$ from the ovine uterus. Endocrinology 95, 1340-1349.

Barley, D.A., Butcher, R.L. & Inskeep, E.K. (1966). Local uterine regulation of luteal regression. Journal of Animal Science 25, 918.

Barrett, S., Blockey, M.A. de B., Brown, J.M., Cumming, I.A., Goding, J.R., Mole, B.J. & Obst, J.M. (1971). Initiation of the oestrous cycle in the ewe by infusions of $PGF_2\alpha$ to the auto-transplanted ovary. Journal of Reproduction & Fertility 24, 136-137.

Bassett, J.M., Oxborrow, T.J., Smith, I.D. & Thorburn, G.D. (1969). The concentration of progesterone in the peripheral plasma of the pregnant ewe. Journal of Endocrinology 45, 449-457.

Bedford, C.A., Harrison, F.A. & Heap, R.B. (1974). Splanchnic, uterine, ovarian and adrenal uptake of progesterone and 20α- dihydroprogesterone in the pregnant and non-pregnant sheep. Journal of Endocrinology 62, 277-290.

Rehrman, H.R., Grinwich, D.L., Hickens, M. & Macdonald, G.J. (1978). Effect of hypophysectomy, prolactin and prostaglandin $F_2\alpha$ on gonadotropin binding in vivo and in vitro in the corpus luteum. Endocrinology 103, 349-357.

Bellvé, A.R. & McDonald, M.F. (1968). Directional flow of fallopian tube secretion in the Romney ewe. Journal of Reproduction & Fertility 15, 357-364.

Binder, D., Bowler, J., Brown, E.D., Crossley, N.S., Hutton, J., Senior, M., Slater, L., Wilkinson, P. & Wright, N.C.A. (1974). 16-Aryloxy prostaglandins: a new class of potent luteolytic agent. Prostaglandins 6, 87-90.

Bindon, B.M., Ch'ang, T.S. & Turner, H.N. (1971). Ovarian response to gonadotrophin by Merino ewes selected for fecundity. Australian Journal of Agricultural Research 22, 809-820.

Bjersing, L., Hay, M.F., Kann, G., Moor, R.M. Naftolin, F., Scaramuzzi, R.J., Short, R.V. & Younglai, E.V. (1972). Changes in gonadotrophins, ovarian steroids and follicular morphology in sheep at oestrus. Journal of Endocrinology 52, 465-479.

Bjersing, L., Hay, M.F., Moor, R.M., Short, R.V. & Deane, H.W. (1970). Endocrine activity, histochemistry and ultrastructure of ovine corpora lutea. 1. Further observations on regression at the end of the oestrous cycle. Zeitschrift für Zellforschung und Mikroskopisch Anatomie 111, 437-457.

Bland, K.P. & Donovan, B.T. (1966). The uterus and the control of ovarian function. Advances in Reproductive Physiology I, 179-214.

Bland, K.P., Horton, E.W. & Poyser, N.L. (1971). Levels of $PGF_2\alpha$ in the uterine venous blood of sheep during the oestrous cycle Life Sciences 10, 509-517.

Blatchley, F.R. & Poyser, N.L. (1974). The effect of oestrogen and progesterone on the release of prostaglandins from the uterus of the ovariectomized guinea-pig. Journal of Reproduction & Fertility 40, 205-209.

Bolt, D.J. & Hawk, H.W. (1972). Failure of exogenous oestrogens to induce CL regression in hysterectomized ewes. Journal of Animal Science 35, 237.

Bolt, D.J. & Hawk, H.W. (1975). Prevention of oestrogen-induced regression of corpora lutea in ewes by hysterectomy. Journal of Animal Science 40, 687-690.

Bolt, D.J., Kelley, H.E. & Hawk, H.W. (1971). Influence of gonadotrophic hormones on oestradiol-induced corpus luteum regression in the ewe. Journal of Animal Science 32, 977-980.

Bolt, D.J. Menzies, C.S. & Spies, H.G. (1966). Influence of pregnancy and hysterectomy on corpora lutea of ewes. Journal of Animal Science 25, 919.

Brand, A.& de Jong, W.H.R. (1973). Qualitative and quantitative micromorphological investigations of the tertiary follicle population during the oestrous cycle in sheep. Journal of Reproduction & Fertility 33, 431-439.

Brinsfield, T.H. & Hawk, H.W. (1967). Luteolytic effect of induced uterine infection in the ewes. Journal of Animal Science 26, 941.

Brinsfield, T.H. & Hawk, H.W. (1968). Luteolytic effect of induced uterine infection in the ewe. Journal of Animal Science 27, 150-152.

Brinsfield, T.H., Hawk, H.W. & Leffel, E.C. (1963). Control by ovarian hormones of the inflammatory response in the sheep uterus. Journal of Reproduction & Fertility 6, 79-86.

Brinsfield, T.H., Higginbotham, D.K. & Hawk, H.W. (1969). Influence of induced uterine infection at various stages of the estrous cycle on the corpus luteum of the ewe. Journal of Animal Science 29, 616-618.

Brown, B.W., Hales, J.R.S. & Mattner, P.E. (1974). Cyclic changes in blood flow in the genital tract of the ewe. Journal of Reproduction & Fertility 36, 490.

Brown, J.M., Catt, K.J., Cumming, I.A., Goding, J.R., Kaltenbach, C.C. & Mole, B.J. (1969). The release of luteinizing hormone in the ewe following oestradiol administration. Journal of Physiology 201, 98p-100p.

Bruce, N.W. & Moor, R.M. (1976). Capillary blood flow to ovarian follicles, stroma and corpora lutea of anaesthetized sheep. Journal of Reproduction & Fertility 46, 299-304.

Bryant, G.D., Greenwood, F.C., Kann, G., Martinet, J. & Denamur, R. (1971). Plasma prolactin in the oestrous cycle of the ewe: effect of pituitary stalk section. Journal of Endocrinology 51, 405-406.

Caffrey, J.L., Nett, T.M., Abel, J.H. & Niswender, G.D. (1979). Activity of 3β-Hydroxy-Δ^5-Steroid Dehydrogenase/Δ^5-Δ^4-Isomerase in the ovine corpus luteum. Biology of Reproduction 20, 279-287.

Caldwell, B.V. (1970). Uterine factors influencing corpus luteum function. Advances in the Biosciences 4, 399-417.

Caldwell, B.V. & Moor, R.M. (1971). Further studies on the role of the uterus in the regulation of corpus luteum function in sheep. Journal of Reproduction & Fertility 26, 133-135.

Caldwell, B.V., Moor, R.M. & Lawson, R.A.S. (1968). Effect of sheep endometrial grafts and extracts on the length of pseudopregnancy in the hysterectomized hamster. Journal of Reproduction & Fertility 17, 567-569.

Caldwell, B.V., Rowson, L.E.A., Moor, R.M. & Hay, M.F. (1969). The utero-ovarian relationship and its possible role in infertility. Journal of Reproduction & Fertility, Suppl. 8, 59-76.

Caldwell, B.V., Tillson, S.A., Brock, W.A. & Speroff, L. (1972). The effects of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes. Prostaglandin 1, 217-228.

Carlson, J.C., Barcikowski, B., Cargill, V. & McCracken, J.A. (1974). The blockade of LH release by indomethacin. Journal of Clinical Endocrinology and Metabolism 39, 399-402.

Carlson, J.C., Barcikowski, B. & McCracken, J.A. (1973). Prostaglandin $F_2\alpha$ and the release of LH in sheep. Journal of Reproduction & Fertility 34, 357-361.

Carlson, J.C., Rugg, A.E., Glew, M.E., Barcikowski, B. & McCracken, J.A. (1972). Luteolytic properties of prostaglandin $F_1\alpha$ in sheep. Biology of Reproduction 7, 109.

Cerini, J.C., Cain, M.D., Chamley, W.A., Cumming, I.A., Findlay, J.K. & Goding, J.R. (1973). Luteolysis in the ewe: a study using a radioimmunoassay for prostaglandin F . Journal of Reproduction & Fertility 32, 326-327.

Cerini, M.E.D., Beck, C., Chamley, W.A., Cumming, I.A., Findlay, J.K. & Goding, J.R. (1973). The effect of an ovine LH and a placental protein on the maintenance of the corpus luteum in the ewe. Journal of Reproduction & Fertility 32, 324-325.

Chamley, W.A., Brown, J.M., Cain, M.D., Cerini, J.C., Cerini, M.E.D., Cumming, I.A., Goding, J.R. & Kragt, C. (1972). Luteolysis following intra-arterial infusion of prostaglandin $F_2\alpha$ directly into the ovine autotransplanted ovary. Journal of Reproduction & Fertility 28, 153-155.

Chamley, W.A., Buckmaster, J.M., Cain, M.D., Cerini, J.C., Cerini, M.E.D., Cumming, I.A. & Goding, J.R. (1972). The effect of prostaglandin $F_{2\alpha}$ on progesterone, oestriol and luteinizing hormone secretion in sheep with autotransplants. Journal of Endocrinology 55, 253-263.

Chamley, W.A., Cerini, J.C., Cerini, M.E., Cumming, I.A., Goding, J.R. & O'Shea, J.D. (1974). Luteal function following the infusion of prostaglandin $F_2\alpha$ into the uterine vein of the ewe. Biology of Reproduction 11, 191-197.

Chamley, W.A., Jonas, H.A. & Parr, R.A. (1976). Content of LH, FSH and growth hormone in the pituitaries of pregnant and anestrous sheep. Endocrinology 98, 1535-1538.

Chamley, W.A. & O'Shea, J.D. (1975). Luteal function in the ewe after injection of $PGF_2\alpha$ directly into the CL. Journal of Reproduction & Fertility 43, 401-402.

Chan, W.K. (1974). Oxytocin-induced release of prostaglandin-like substances in isolated rat uterus. Life Science 14, 2385-2392.

Channing, C.P. (1966). Progesterone biosynthesis by equine granulosa cells growing in tissue culture. Nature, London, 210, 1266.

Channing, C.P. (1969). The use of tissue culture of granulosa cells as a method of studying the mechanism of luteinization. In: The Gonads. K.W. McKern Ed., Appleton-Century-Crofts, New York, Chap. 9, 245-275.

Collett, R.A., Land, R.B. & Baird, D.T. (1973). The patern of progesterone secretion by the autotransplanted ovary of the ewe in response to ovine luteinizing hormone. Journal of Endocrinology 56, 403-411.

Collins, W.E., Inskeep, E.K., Howland, B.E., Pope, A.L., & Casida, L.E. (1966). Effects of hysterectomy and corpus luteum induction on pituitary-ovarian relationships in the ewe. Journal of Animal Science 25, 87-91.

Cook, B., Kaltenbach, C.C., Niswender, G.D., Norton, H.W. & Nalbandoc, A.V. (1969). Short term ovarian response to some pituitary hormones infused in vivo in pigs and sheep. Journal of Animal Science 29, 711-718.

Coudert, S.P., Phillips, G.D., Faiman, C., Chernecki, W. & Palmer, M. (1974a). A study of the utero-ovarian circulation in sheep with reference to local transfer between venous and arterial blood. Journal of Reproduction & Fertility 36, 319-331.

Coudert, S.P., Phillips, G.D., Faiman, C., Chernecki, W. & Palmer, M. (1974b). Infusion of tritiated prostaglandin $F_2\alpha$ into the

anterior uterine vein of the ewe: absence of local venous-arterial transfer. Journal of Reproduction & Fertility 36, 333-343.

Coudert, S.P., Phillips, G.D., Palmer,M.& Faiman, C. (1972). Prostaglandin F concentration in the peripheral blood of the ewe during estrous cycle. Prostaglandins 2, 501-509.

Coudert, S.P. & Short, R.V. (1966). Prolongation of the functional life of the corpus luteum in sheep with experimental uterine infection. Journal of Reproduction & Fertility 12, 579-582.

Coudert, S.P. & Short, R.V. (1968). The effects of chlorpromazin treatment on the secretory activity of corpora lutea in hysterectomized sheep. Journal of Reproduction & Fertility 16, 151-154.

Cowie, A.T. (1966). Anterior pituitary function in lactation. In: The pituitary gland, Eds. G.W. Harris & B.T. Donovan, London, Butterworths. Vol 2, Anterior Pituitary, p 412-443.

Cox, R.I., Mattner, P.E. & Thorburn, G.D. (1971). Changes in ovarian secretion of oestradiol-17β around oestrus in the sheep. Journal of Endocrinology 49, 345-346.

Cox, R.I., Thorburn, G.D., Currie, W.B. & Restall, B.J. (1974). Interrelations between oestrogens and prostaglandin F concentrations in the utero-ovarian venous plasma of the ewe. Journal of Reproduction & Fertility 36, 448-449.

Cox, R.I., Thorburn, G.D., Currie, W.B. Restall, B.J. & Schneider, W. (1973). Prostaglandin F group (PGF), progesterone, and estrogen concentrations in the uteroovarian venous plasma of the conscious ewe during the estrous cycle. Advances in the Biosciences 9, 625-630.

Cumming, I.A., Baxter, R.W., Buckmaster, J.M., Jonas, H.A., Findlay, J.K. & Goding J.R. (1974). Control of FSH secretion in the ewe. Journal of Reproduction & Fertility 36, 447-448.

Cumming, I.A., Brown, J.M., Goding, J.R., Bryant, G.D. & Greenwood, F.C. (1972). Secretion of prolactin and luteinizing hormone at oestrus in the ewe. Journal of Endocrinology 54, 207-213.

Davis , S.L., Reichert, L.E. & Niswender, G.D. (1971). Serum levels of prolactin in sheep as measured by radioimmunoassay. Biology of Reproduction 4, 145-153.

Deane, H.W., Hay, M.F., Moor, R.M., Rowson, L.E.A. & Short, R.V. (1966). The corpus luteum of the sheep : relationships between morphology and function during the oestrous cycle. Acta endocrinology 51, 245-263.

De Angelo, A.B. & Gorski, J. (1970). Role of RNA synthesis in the estrogen induction of a specific uterine protein. Proceedings of the National Academy of Sciences of the United States of America 66, 693-700.

Del Campo, C.H. & Ginther, O.J. (1973a). Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: Horses, sheep and swine. American Journal of Veterinary Research 34, 305-316.

Del Campo, C.H. & Ginther, O.J. (1973b). Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: Angio-architecture in sheep. American Journal of Veterinary Research 34, 1377-1385.

Del Campo, C.H. & Ginther, O.J. (1974). Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: Histological structure of utero-ovarian vein and ovarian artery in sheep. American Journal of Veterinary Research 35, 397-399.

Denamur, R. (1968). Formation and maintenance of corpora lutea in domestic Animals. Journal of Animal Science 27, Suppl. 1, 163-180.

Denamur, R. (1974). Luteotrophic factors in sheep. Journal of Reproduction & Fertility 38, 251-259.

Denamur, R. & Kann, G. (1973). Luteolytic effects of oestradiol after hypophysectomy or pituitary stalk section in cycling sheep. Acta endocrinologica 73, 635-642.

Denamur, R., Martinet, J. & Short, R.V. (1966a). Secretion de la progesterone par les corps jaunes de la brebis après hypophysectomie, section de la tige pituitaire et hysterectomie. Acta endocrinologica 52, 72-90.

Denamur, R., Martinet, J. & Short, R.V. (1966b). Luteal maintenance in hypophysectomized and hysterectomized sheep. Quoted by Thibault, C. (1966). Journal of Reproduction & Fertility, Suppl. 1, 63-65.

Denamur, R., Martinet, J. & Short, R.V. (1970). Mode of action of oestrogen in maintaining the functional life of the corpora lutea in sheep. Journal of Reproduction & Fertility. 23, 109-116.

Denamur, R. Martinet, J. & Short, R.V. (1973). Pituitary control of the ovine corpus luteum. Journal of Reproduction & Fertility 32, 207-220.

Denamur, R. & Mauléon, P. (1963a). Effects de l'hypophysectomie sur la morphologie et l'histologie du corps jaune des ovins. Compte rendu hebdomadaire des séances de l'Academie des sciences 257, 264-268.

Denamur, R. & Mauléon, P. (1963b). Contrôle endocrinien de la persistance du corps jaune chez les ovins. Compte rendu hebdomandaire des séances de l'Académie des sciences 257, 527-530.

Denamur, R. & Short, R.V. (1973). Some aspects of the luteotropic control of the ovine corpus luteum. In: The Regulation of Mammalian Reproduction, p 441-446. Eds. S.J. Segal, R. Crozier, P.A. Corfman, & P.G. Condliffe, Charles C. Thomas, Illinois, U.S.A.

Diekman, M.A., O'Callaghan, P., Nett, T.M. & Niswender, G.D. (1978a). Effect of prostaglandin $F_{2\alpha}$ on the number of LH receptors in ovine corpora lutea. Biology of Reproduction 19, 1010-1013.

Diekman, M.A., O'Callaghan, P., Nett, T.M. & Niswender, G.D. (1978b). Validation of methods and quantification of luteal receptors for LH throughout the estrous cycle and early pregnancy in ewe. Biology of Reproduction 19, 999-1009.

Dighe, K.K., Emslie, H.A., Henderson, L.K., Rutherford, F. & Simon, L.(1975). The development of antisera to prostaglandin B_2 and $F_{2\alpha}$ and their analysis using solid-phase and double antibody radioimmunoassay methods. British Journal of Pharmacology 55, 503-514.

Dobrowolski, W. & Hafez, E.S. (1970). Ovariouterine vasculature in sheep. American Journal of Veterinary Research 31, 2121-2126.

Domański, E., Skrzeczkowski, L., Stupnicka, E., Fitko, R. & Dobrowolski, W. (1967). Effect of gonadotrophins on the secretion of progesterone and oestrogens by the sheep ovary perfused in situ.

Journal of Reproduction & Fertility 14, 365-372.

Douglas, R.H. & Ginther, O.J. (1973). Luteolysis following a single injection of prostaglandin $F_2\alpha$ in sheep. Journal of Animal Science 37, 990-993.

Dufour, J., Ginther, O.J. & Casida, L.E. (1971a). Response of ovaries to removal of corpora lutea in sheep. Journal of Animal Science 32, 1202-1205.

Dufour, J.J., Ginther, O.J. & Casida, L.E. (1971b). Corpus luteum action on ovarian follicular development after destruction of macroscopically visible follicles in ewes. Proceedings of the Society for Experimental Biology and Medicine 138, 475-478.

Dufour, J., Ginther, O.J. & Casida, L.E. (1972). Intra-ovarian relationship between corpora lutea and ovarian follicles in ewes. American Journal of Veterinary Research 33, 1445-1446.

Dutt, R.H. & Casida, L.E. (1948). Alteration of the estrual cycle in sheep by use of progesterone and its effect upon subsequent ovulation and fertility. Endocrinology 43, 208-217.

Eastwood, K.C., Payne, E., Fairclough, R.J. & McDonald, M.F. (1976). Plasma progesterone concentration during pregnancy in Romney and Border Leicester x Romney ewes after gonadotrophin treatment. New Zealand Journal of Agricultural Research 19, 271-276.

Edgar, D.G. & Ronaldson, J.W. (1958). Blood levels of progesterone in the ewe. Journal of Endocrinology 16, 378-384.

Einer-Jensen, N. & McCracken, J.A. (1976). Krypton measurement of capillary blood flow in the ovine corpus luteum during $PGF_2\alpha$-induced luteolysis. In: Recent Advances in Prostaglandin and Thromboxane Research, Eds. R. Paoletti & B. Samuelsson, New York, Raven, Vol 2., p 901.

Eliasson, R. (1959). Studies on prostaglandin occurrence, formation and biological actions. Acta Physiologica Scandinavica 46, Suppl. 158, 1-73.

Euler, U.S. von (1934). Zur kenntnis der pharmakologischen Wirkungen von nativsekreten und extrakten männticher accessorischer geschlechtsdrüsen. Archiv für experimentelle Pathologie und Pharmakologie 175, 78-84.

Euler, U.S. von (1935). A depressor substance in the vesicular gland. Journal of Physiology 84 21p-22p.

Euler, U.S. von (1936). On the specific vasodilating and plain muscle stimulating substances from accessory genital glands in man and certain animals. (Prostaglandin and vesiglandin). Journal of Physiology 88, 213-234.

Euler, U.S. von & Hammarstöm, S. (1937). Uber das vorkommen des prostaglandins in tierorganes. Skandinavisches Archiv für Physiologie 77, 96-99.

Fairclough, R.J., Smith, J.F., Peterson, A.J. & McGowan, L. (1976). Effect of oestradiol-17β , progesterone and prostaglandin $F_2\alpha$ antiplasma on luteal function in the ewe. Journal of Reproduction & Fertility 46, 523-524.

Fenwick, L., Jones, R.L., Naylor, E., Poyser, N.L. & Wilson, N.H (1977). Production of prostaglandins by the pseudo-pregnant rat uterus in vitro, and the effect to taxifen with the identification of 6-keto-prostaglandin $F_1\alpha$ as a major product. British Journal of Pharmacology 59, 191-199.

Ferreira, S.H. & Vane, J.R. (1967). Prostaglandins: their disappearance from and release into the circulation. Nature, London 216, 868.

Fitzpatrick, R.J. & Sharman, S.C. (1973). Prostaglandin F in the vena caval blood during the oestrous cycle of the ewe. Journal of Physiology 234, 57p-59p.

Fogwell, R.L., Lewis, G.S., Butcher, R.L. & Inskeep, E.K. (1977). Effects of ovarian bisection on response to intra-follicular injection of $PGF_2\alpha$ and on follicular development in ewes. Journal of Animal Science 45, 328-335.

Foote, W.C. (1964). Influence of estradiol and progesterone on corpus luteum formation and maintenance in sheep. Journal of Animal Science 23, 905.

Frobes, Y.M. & Clegg, M.T. (1969). Plasma and pituitary FSH and LH in ewes bearing an intrauterine device. 2nd Annual Meeting of the society for the study of reproduction, Davis, California (Abstr. 4), p 2.

Ford, S.P. Pexton, J.E., Wilson, L., Butcher, R.L. & Inskeep, E.K. (1973). Effects of estradiol-17β on uterine PGF in ewes. Journal of Animal Science 37, 311.

Ford, S.P., Weems, C.W., Pitts, R.E., Pexton, J.E., Butcher, R.L. & Inskeep, E.K. (1975). Effect of estradiol-17β and progesterone on PGF in sheep uteri and uterine venous plasma. Journal of Animal Science 41, 1407-1413.

French, L.R. (1973). Modification of the uterine luteolytic functin. Ph.D. Thesis, University of Wisconsin, Medison.

French, L.R. & Casida, L.E. (1973). Effect of actinomycin D on corpus luteum regression in ewes. Journal of Animal Science 37, 1218-1221.

Fuller, G.B. & Hansel, W. (1970). Regression of sheep corpora lutea after treatment with antibovine luteinizing hormone. Journal of Animal Science 31, 99-103.

Fylling, P. (1970). The effect of pregnancy, ovariectomy and parturition on plasma progesterone level in sheep. Acta endocrinologica. 65, 273-283.

Gemmell, R.T., Stacy, B.D., & Thorburn, G.D. (1974a). The ultrastructure of the corpus luteum in relation to the mechanism of progesterone secretion. Journal of Reproduction & Fertility 36, 444-445.

Gemmell, R.T., Stacy, B.D. & Thorburn, G.D. (1974b). Ultrastructure of secretory granules in the corpus luteum of the sheep during the estrous cycle. Biology of Reproduction 11, 447-462.

Gemmell, R.T., Stacy, B.D. & Thorburn, G.D. (1976). Morphology of the regressing corpus luteum in the ewe. Biology of Reproduction 14, 270-279.

Gengenbach, D.R., Hixon, J.E. & Hansel, W. (1977). A luteolytic interaction between estradiol and prostaglandin $F_2\alpha$ in hysterectomized ewes. Biology of Reproduction 16, 571-579.

Geschwind, I.I. (1972). Dynamics of pituitary gonadotropin secretion. Journal of Animal Science 34, Suppl. 1, 19-38.

Ginther, O.J. (1968a). Influence of an intrauterine device on ovarian activity in the ewe. Journal of Animal Science 27, 1611-1613.

Ginther, O.J. (1968b). Influence of exogenous progesterone and the uterus on ovarian activity in sheep. Endocrinology 83, 613-615.

Ginther, O.J. (1969). Length of estrous cycle and size at corpus luteum in guinea pigs and sheep treated with progesterone at different days of the estrous cycle. American Journal of Veterinary Research 30, 1975-1978.

Ginther, O.J. (1970). Effect of unilateral hysterectomy and separation and ligation of uterine horns on luteolytic activity of an intrauterine device in sheep. American Journal of Veterinary Research 31, 2127-2130.

Ginther, O.J. (1971). Response of corpora lutea to cauterization of follicles in sheep. American Journal of Veterinary Research 32, 59-62.

Ginther, O.J. (1974). Internal regulation of physiological processes through local veno-arterial pathways: A review. Journal of Animal Science 39, 550-564.

Ginther, O.J. & Bisgard, G.E. (1972). Role of main uterine vein in local action of an intrauterine device on the corpus luteum in sheep. American Journal of Veterinary Research 33, 1583-1587.

Ginther, O.J. & Del Campo, C.H. (1973). Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: Area of close apposition between the ovarian artery and vessels which contain uterine venous blood in sheep. American Journal of Veterinary Research 34, 1387-1393.

Ginther, O.J., Del Campo, C.H. & Rawlings, C.A. (1973). Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: A local venoarterial pathway between the uterus and ovaries in sheep. American Journal of Veterinary Research 34, 723-728.

Ginther, O.J., Hawk, H.W. & Casida, L.E. (1966). Pituitary LH activity of ewes treated with an intrauterine device. Journal of Animal Science 25, 1262.

Ginther, O.J., Pope, A.L. & Casida, L.E. (1965). Some effects of intrauterine plastic coils in ewes. Journal of Animal Science 24, 918-919.

Ginther, O.J., Pope, A.L. & Casida, L.E. (1966). Local effect of an intrauterine plastic coil on the corpus luteum of the ewe. Journal of Animal Science 25, 472-475.

Ginther, O.J., Woody, C.O., Mahajan, S., Jarakiraman, K. & Casida, L.E. (1967). Effect of oxytocin administration on the oestrous cycle of unilaterally hysterectomized heifers. Journal of Reproduction & Fertility 14, 225-229.

Goding, J.R. (1966). Ovarian autotransplantation with vascular anastomoses, and its application to the study of reproductive physiology in the ewe. Journal of Physiology 186, 86p-87p.

Goding, J.R. (1973). The demonstration that $PGF_{2\alpha}$ is the uterine luteolysin in the ewe. In: Le Corps Jaune Eds. R. Denamur & A. Natter, Colloque de la société nationale pour l'étude de la stérilité et de la fécondité, Masson et Cie Editeurs, p 311-323.

Goding, J.R. (1974). The demonstration that $PGF_2\alpha$ is the uterine luteolysin in the ewe. Journal of Reproduction & Fertility 38, 261-271.

Goding, J.R., Baird, D.T., Cumming, I.A. & McCracken, J.A. (1971). Functional assessment of autotransplanted endocrine organs. In: Perfusion Techniques, p 169-199. Karolinska symposia on Research Methods in Reproductive Technology.

Goding, J.R., Blockey, M.A., Brown, J.M., Catt, K.J. & Cumming, I.A. (1970). The role of oestrogen in the control of the oestrous cycle in the ewe. Journal of Reproduction & Fertility 21, 268-369.

Goding, J.R, Cain, M.D., Cerini, J., Cerini, M., Chamley, W.A. & Cumming, I.A. (1972). Prostaglandin $F_2\alpha$ "the"luteolytic hormone in the ewe. Journal of Reproduction & Fertility 28, 146-147.

Goding, J.R., Catt, K.J., Brown, J.M., Kaltenbach, C.C., Cumming, I.A. & Mole, B.J. (1969). Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep. Endocrinology 85, 133-142.

Goding, J.R., Harrison, F.A., Heap, R.B. & Linzell, J.L. (1967). Ovarian activity in the ewe after autotransplantation of the ovary or uterus to the neck. Journal of Physiology 191, 129p-130p.

Goding, J.R., McCracken, J.A. & Baird, D. (1967a). The secretion of steroids in ovarian venous blood of the ewe. Proceedings of the Third Asia and Oceania Congress of Endocrinology, Manila, p 701.

Goding, J.R., McCracken, J.A. & Baird, D.T. (1967b). The study of ovarian function in the ewe by means of a vascular autotransplantation technique. Journal of Endocrinology 39, 37-52.

Goldberg, V.J. & Ramwell, P.W. (1975). Role of prostaglandins in reproduction. Physiological Reviews 55, 325-351.

Goldblatt, M.W. (1933). A depressor substance in seminal fluid. Journal of the Society of Chemical Industry 52, 1056-1057.

Goldblatt, M.W. (1935). Properties of human seminal plasma. Journal of Physiology 84, 208-218.

Goodman, R.L. (1978). Role of ovarian steroids in the initiation and synchronization of behavioural estrus and the LH surge in the ewe. Eleventh Annual Meeting of the society for the study of reproduction. Biology of Reproduction 18, Suppl. 1 46A.

Goodman, R.L. & Karsch, F.J. (1979). Do oestradiol and progesterone act at different sites to inhibit tonic LH secretion during the breeding season of the ewe? Programme and Abstracts of Papers of the Annual Conference of Society for the Study of Fertility, University of Glasgow, p 35.

Gordon, I. (1958). The use of progesterone and serum gonadotrophin (P.M.S.) in the control of fertility in sheep. II. studies in the extra-seasonal production of lambs. Journal of Agricultural Science 50, 152-197.

Grant, R. (1934). Studies on the physiology of reproduction in the ewe. Part I. The symptoms, periodicity, and duration of oestrus. Part II. Changes in the vagina and cervix. Part III. Gross changes in the ovaries. Transactions of the Royal Society of Edinburgh 58, 1-47.

Gréen, K., Samuelsson, B., Carlson, J.C. & McCracken, J.A. (1971). Quoted by McCracken, J.A., Baird, D.T. & Goding, J.R. (1971).

Greenwood, B., & Kerry, P.J. (1975). Prostaglandin production by a mild inflammatory lesion in sheep. British Journal of Pharmacology 53, 305-307.

Ham, E.A., Cirillo, V.J., Zanetti, M.E. & Kuehl, F.A. (1975). Estrogen-directed synthesis of specific prostaglandins in the uterus. Proceedings of the National Academy of Sciences of the United States of America 72, 1420-1424.

Hansel, W. (1969). Lutoelysin transfer from uterine vein to the ovarian artery. Quoted by McCracken, J.A., Barcikowski, B., Carlson J.C., Gréen, K. & Samuelsson, B. (1973). Advances in Biosciences 9, 599-624.

Hansel, W. & Wagner, W.C. (1960). Luteal inhibition of the bovine as a result of oxytocin injections, uterine dilation and intrauterine infusions of seminal and preputial fluids. Journal of Dairy Science 43, 796-805.

Haresign, W. & Lamming, G.E. (1978). Comparison of LH release and luteal function in cyclic and LH-RH-treated anoestrous ewes pretreated with PMSG or oestrogen. Journal of Reproduction & Fertility 52, 349-353.

Harrison, F.A., Heap, R.B., Horton, E.W. & Poyser, N.L. (1972). Identification of prostaglandin $F_2\alpha$ in uterine fluid from the non-pregnant sheep with an autotransplanted ovary. Journal of Endocrinology 53, 215-222.

Harrison, F.A., Heap, R.B. & Linzell, J.L. (1968). Ovarian function in the sheep after autotransplantation of the ovary and uterus to the neck. Journal of Endocrinology 40, xiii.

Harrison, F.A., Heap, R.B. & Poyser, N.L. (1976). Production, chemical composition and prostaglandin $F_2\alpha$ content of uterine fluid in pregnant sheep. Journal of Reproduction & Fertility 48, 61-67.

Hauger, R.L., Karsch, F.J. & Foster, D.L. (1977). A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. Endocrinology 101, 807-817.

Hawk, H.W. & Bolt, D.J. (1970). Luteolytic effect of oestradiol-17β when administered after mid cycle in the ewe. Biology of Reproduction 2, 275-278.

Hawk, H.W., Brinsfield, T.H. & Righter, H.F. (1963). Control by ovarian hormones of vascular permeability in normal and experimentally infected sheep. Journal of Reproduction & Fertility 6, 71-77.

Hawk, H.W., Turner, G.D. & Sykes, J.F. (1961). Variation in the inflammatory response and bactericidal activity of the sheep uterus during the oestrous cycle. American Journal of Veterinary Research 22, 689-692.

Heap, R.B. & Poyser, N.L. (1975). Prostaglandins in pyometrial fluid from the cow, bitch and ferret. British Journal of Pharmacology 55, 515-518.

Hearnshaw, J., Restall, B.J. & Gleeson, A.R. (1973). Observations on the luteolytic effects of prostaglandin $F_2\alpha$ during the oestrous cycle and early pregnancy in the ewe. Journal of Reproduction & Fertility 32, 322-323.

Hecker, J.F., Bray, A.R. & Wodzicka-Tomaszewska, M. (1974). Effect of exogenous progesterone on the ovarian cycle of ewe. Journal of Reproduction & Fertility 36, 485.

Henderson, K.M. & McNatty, K.P. (1975). A biochemical hypothesis to explain the mechanism of luteal regression. Prostaglandins 9, 779-797.

Henderson, K.M. & McNatty, K.P. (1977). A possible inter-relationship between gonadotrophin stimulation and prostaglandin $F_2\alpha$ inhibition of steroidogenesis by granulosa-luteal cells *in vitro*. Journal of Endocrinology 73, 71-78.

Henderson, K.M., Scaramuzzi, R.J. & Baird, D.T. (1977). Simultaneous infusion of prostaglandin E_2 antagonizes the luteolytic action of prostaglandin $F_2\alpha$ *in vivo*. Journal of Endocrinology 72, 379-383.

Hertelendy, F., Todd, H., Ehrhart, K. & Blute, R. (1972). Studies on growth hormone secretion: IV. In vivo effects of prostaglandin E_1. Prostaglandins 2, 79-91.

Hixon, J.E. & Clegg, M.T. (1969). Influence of pituitary on ovarian progesterone output in the ewe. Effects of hypophysectomy and gonadotropic hormones. Endocrinology 84, 828-834.

Hixon, J.E., Gengenbach, D.R. & Hansel, W. (1975). Failure of prostaglandin $F_2\alpha$ to cause luteal regression in ewes after destruction of ovarian follicles by X-irradiation. Biology of Reproduction 13, 126-135.

Holst, P.J., Barden, W.H. & Mattner, P.E. (1972). Oestradiol-17β secretion from the ewe ovary and related ovarian morphology on day 2 and 3 of the cycle. Journal of Reproduction & Fertility 28, 136.

Hopkinson, C.R.N. & Pant, H.C. (1973). Heterologous radioimmunoassay of circulating ovine FSH in normal ewes. Journal of Physiology 231, 52p-53p.

Horton, E.W. & Poyser, N.L. (1976). Uterine luteolytic hormone: A physiology role for prostaglandin $F_2\alpha$. Physiological Review 56, 595-651.

Howland, B.E., Kirkpatrick, R.L., Woody, C.O., Pope, A.C. & Casida, L.E. (1968). Effects of a single injection of oestrogen early in the estrous cycle on pituitary and luteal function in ewes. Journal of Animal Science 27, 1401-1403.

Hutchinson, J.S.M. & Robertson, H.A. (1966). The growth of the follicle and corpus luteum in the ovary of the sheep. Research in Veterinary Science 7, 17-24.

Inskeep, E.K., & Butcher, R.L. (1966). Local components of utero-ovarian relationships in the ewe. Journal of Animal Science 25, 1164-1168.

Inskeep, E.K., Howland, B.E., Pope, A.L. & Casida, L.E. (1964a). Some effects of progesterone on experimentally induced corpora lutea in ewes. Journal of Animal Science 23, 791-794.

Inskeep, E.K., Howland, E.B., Pope, A.L. & Casida, L.E. (1964b). Ability of corpora lutea induced experimentally in progesterone treated ewes to prevent subsequent ovulation. Journal of Animal Science 23, 1172-1175.

Inskeep,E.K.,Oloufa,M.M.,Howland, B.E., Pope, A.L. & Casida, L.E. (1962). Effect of experimental uterine distention on estrual cycle lengths in ewes. Journal of Animal Science 21, 331-332.

Inskeep, E.K., Oloufa, M.M., Pope, A.L. & Casida, L.E. (1963). Functional capabilities of experimentally induced corpora lutea in ewes. Journal of Animal Science 22, 159-161.

Inskeep, E.K., Smutny, W.J., Butcher, R.L. & Pexton, J.E. (1975). Effects of intrafollicular injections of prostaglandins in non-pregnant ewes. Journal of Animal Science 41, 1098-1104.

International Committee of Veterinary Anatomical Nomenculature, Nomina Anatomica Veterinaria (1968). International Committee on Veterinary Anatomical Nomenclature, Vienna.

Jonas, H.A., Salamonsen, L.A., Burger, H.G., Chamley, W.A., Cumming, I.A., Findlay, J.K. & Goding, J.R. (1973). Release of FSH after administration of gonadotrophin-releasing hormone or estradiol to the anestrous ewe. Endocrinology 92, 862-865.

Jones, R.L., Poyser, N.L. & Wilson, N.H. (1977). Production of 6-oxo-prostaglandin $F_{1\alpha}$ by rat, guinea-pig and sheep uteri *in vitro*. British Journal of Pharmacology 59, 436P-437P.

Kaltenbach, C.C. (1968). Discussion. Journal of Animal Science 27, Suppl. 1, 204-206.

Kaltenbach, C.C., Cook, B., Niswender, G.D. & Nalbandov, A.V. (1966). Progesterone synthesis by ovine luteal tissue *in vitro*. Journal of Animal Science 25, 926.

Kaltenbach, C.C., Cook, B., Niswender, G.D. & Nalbandov, A.V., (1967). Effect of pituitary hormones on progesterone synthesis by ovine luteal tissue in vitro, Endocrinology 81, 1407-1409.

Kaltenbach, C.C., Graber, J.W., Niswender, G.D. & Nalbandov, A.V. (1968a) Effect of hypophysectomy on the formation and maintenance of corpora lutea in the ewe. Endocrinology 82, 753-759.

Kaltenbach, C.C., Graber, J.W., Niswender, G.D. & Nalbandov, A.V. (1968b). Luteotrophic properties of some pituitary hormones in non-pregnant or pregnant hypophysectomized ewes. Endocrinology 82, 818-824.

Kaltenbach, C.C., Niswender, G.D., Graber, J.W. & Nalbandov, A.V. (1966). Corpus luteum function in hypophysectomized ewes. 48th Meeting of the Endocrine Socitey. p 82.

Kann, G. (1971). Variations des concentrations plasmatiques de l'hormone lutéinisante et de la prolactine au cours du cycle oestrien de la brebis. Compte rendu hebdomadaire des séances de l'Academie des Sciences 272, 2934-2937.

Kann, G. & Denamur, R. (1973). Changes in plasma levels of prolactin and LH induced by luteolytic or luteotrophin treatment in intact cycling sheep after secretion of the pituitary stalk. Acta endocrinologica 73, 625-634.

Kann, G. & Denamur, R. (1974). Possible role of prolactin during the oestrous cycle and gestation in the ewe. Journal of Reproduction & Fertility 39, 473-483.

Karim, S.M.M. (1972). The Prostaglandins. Medical and Technical Publishing Co. Ltd., Oxford and Lancaster.

Karsch, F.J., Cook, B., Ellicott, A.R., Foster, D.L., Jackson, G.J. & Nalbandov, A.V. (1971). Failure of infused prolactin to prolong the life-span of the corpus luteum of the ewe. Endocrinology 89, 272-275.

Karsch, F.J. & Legan, S.J. (1978). Roles of progesterone and estradiol in controlling LH secretion during the estrous cycle of the ewe. Eleventh Annual Meeting of the Society for the Study of Reproduction. Biology of Reproduction 18, Suppl. 1, 50A.

Karsch, F.J., Legan, S.J., Hauger, R.L. & Foster, D.L. (1977). Negative feedback action of progesterone on tonic luteinizing hormone

secretion in the ewe: Dependence on the ovaries. Endocrinology 101, 800-806.

Karsch, F.J., Noveroske, J.W., Roche, J.F. & Nalbandov, A.V. (1969a). Response to infused LH depends on age of ovine corpora lutea. Journal of Animal Science 29, 192.

Karsch, F.J., Noveroske, J.W., Roche, J.F. & Nalbandov, A.V. (1969b). Minimal dose of LH for luteal maintenance in ewes. Journal of Animal Science 29, 192.

Karsch, F.J., Noveroske, J.W., Roche, J.F., Norton, H.W. & Nalbandov, A.V. (1970). Maintenance of ovine corpora lutea in the absence of ovarian follicles. Endocrinology 87, 1228-1236.

Karsch, F.J., Roche, J.F., Noveroske, J.W., Foster, D.L., Norton, H.W. & Nalbandov, A.V. (1971). Prolonged maintenance of the corpus luteum of the ewe by continuous infusion of luteinizing hormone. Biology of Reproduction 4, 129-135.

Kerdelhué, B., Kann, G. & Jutisz, M. (1972). Dosage radioimmunologique de la FSH chez mouton et le rat. In: Hormones Glycoprotéiniques Hypophysaires, p 177-192. I.N.S.E.R.M., Paris, Quoted by Denamur, R. (1974).

Kimball, F.A. & Wyngarden, L.J. (1977). Prostaglandin $F_2\alpha$ specific binding in equine corpora lutea. Prostaglandins 13, 553-564.

Kiracofe, G.H. (1966). Effects of uterine extracts, induced ovulation or parabiosis on corpora lutea of hysterectomized sheep and rats. Ph.D. Thesis, Kansas State University (Diss. Abstr. 30th June 1966, p 7414-7415).

Kiracofe, G.H., Menzies, G.S., Gier, H.T. & Spies, H.G. (1966). Effect of uterine extracts and uterine or ovarian blood vessel ligations on ovarian functions of ewes. Journal of Animal Science 25, 1159-1163.

Kiracofe, G.H. & Spies, H.G. (1963). Length of corpus luteum maintenance in hysterctomized ewes. Journal of Animal Science 22, 862.

Kiracofe, G.H. & Spies, H.G. (1964). Late luteal hysterectomy and uterine extracts in ewes. Journal of Animal Science 23, 908.

Kiracofe, G.H., Spies, H.G. & Gier, H.T. (1963). Effect of uterine extracts and ligations in hysterectomized ewes. Journal of Animal Science 22, 862.

Lahav, M., Frend, A & Lindner, H.R. (1976). Abrogation by prostaglandin $F_2\alpha$ of LH-Stimulated cyclic AMP accumulation in isolated rat corpora lutea of pregnancy. Biochemical and Biophysical Communication 68 1294-1300.

Lamond, D.R. & Drost, M. (1973). The counter-current transfer of prostaglandin in the ewe. Prostaglandins 3, 691-695.

Lamond, D.R., Gaddy, R.G. & Kennedy, S.W. (1972). Influence of season and nutrition on luteal plasma progesterone in Rambouillet ewes. Journal of Animal Science 34, 626-629.

Land, R.B., Baird, D.T. & Scaramuzzi, R.J. (1976). Dynamic studies of prostaglandin $F_2\alpha$ in the utero-ovarian circulation of the sheep. Journal of Reproduction & Fertility 47, 209-214.

Land, R.B., Carr, W.R. & Thompson, R. (1979). Genetic and environmental variation in the LH response of ovariectomized sheep to LH-RH. Journal of Reproduction & Fertility 56, 243-248.

Land, R.B., Collett, R.A. & Baird, D.T. (1974). Insensitivity of the autotransplanted ovary of the ewe to ovine luteinizing hormone. Journal of Endocrinology 62, 165-166.

Land, R.B., Pelletier, J., Thimonier, J. & Mauléon, P. (1973). A quantitative study of genetic differencesin the incidence of oestrus,ovulation and plasma luteinizing hormone concentration in the sheep. Journal of Endocrinology 58, 305-317.

Land, R.B., Wheeler, A.G. & Carr, W.R. (1976). Seasonal variation in the oestrogen induced LH discharge of ovariectomized Finnish Landrace and Scottish Blackface ewes. Annales de biologie animal biochimie et biophysique 16, 521-528.

Lee, C.S. & O'Shea, J.D. (1974). Observations on the utero-ovarian vasculature of the sheep. Journal of Reproduction & Fertility 36, 459-460.

Lee, C.S. & O'Shea, J.D. (1975). The extrinsic blood vessels of the ovary of the sheep. Journal of Morphology 148, 287-304.

Legan, S.J., Karsch, F.J. & Foster, D.L. (1977). The endocrine control of seasonal reproductive function in the ewe: A marked change in response to the negative feedback action of estradiol on luteinizing hormone secretion. Endocrinology 101, 818-824.

Legault-Démare, J., Mauléon, P., & Suarez-Soto, M. (1960). Etude de l'activité biologique in vitro des hormones gondotropes. II. stimulation in vitro du corps jaune de brebis par l'hormone gonadotrope sérieque dejument gravide (PMSG). Acta endocrinologica 34, 163-168.

Lewis, G.S., Fogwell, R.L., Thayne, W.V., & Inskeep, E.K. (1976). Variation in uterine venous PGF in ewes. Journal of Animal Science 42, 1577-1578.

Lewis, G.S., Jenkins, P.E., Fogwell, R.L. & Inskeep, E.K. (1978). Concentrations of prostaglandins E_2 and $F_2\alpha$ and their relationship to luteal function in early pregnant ewes. Journal of Animal Science 47, 1314-1323.

Lewis, G.S., Wilson, L., Wilks, J.W., Pexton, J.E., Fogwell, R.L., Ford, S.P., Butcher, R.L., Thayne, W.V. & Inskeep, E.K. (1977). $PGF_2\alpha$ and its metabolites in uterine and jugular venous plasma and endometrium of ewes during early pregnancy. Journal of Animal Science 45, 320-327.

Lewis, P.E., Taylor, W.C. & Inskeep, E.K., (1968). Exogenous progesterone on ovine corpora lutea. Journal of Animal Science 27, 1193.

Lewis, P.E. & Warren, J.E. (1975). Indomethacin inhibits luteolysis in the ewe. Journal of Animal Science 41, 365-366.

L'Hermite, M., Niswender, G.D., Reichert, L.E. & Midgely, A.R. (1972). Serum follicle-stimulating hormone in sheep as measured by radioimmunoassay. Biology of Reproduction 6, 325-332.

Lindner, H.R., Sass, M.B. & Morris, B. (1964). Steroids in the ovarian lymph and blood of conscious ewes. Journal of Endocrinology 30, 361-376.

Linzell, J.L. (1960). Valvular incompetence in the venous drainage of the udder. Journal of Physiology 153, 481-491.

Mapletoft, R.J., Miller, K.F. & Ginther, O.J. (1977). Effects of $PGF_2\alpha$ and PGE_2 on corpora lutea in ewes. Proceedings of the 69th Annual Meeting of the American Society of Animal Science, p 185.

Mattner, P.E. & Braden, A.W.H. (1972). Secretion of oestradiol-17β by the ovine ovary during the luteal phase of the oestrous cycle in relation to ovulation. Journal of Reproduction & Fertility 28, 136-137.

Mattner, P.E., Hales, J.R.S. & Brown, B.W. (1972). Blood flow in the ovary in sheep. Proceedings of Australian Physiological and Pharmacological Society 3, 144.

Mattner, P.E. & Thorburn, G.D. (1969). Ovarian blood flow in sheep during the oestrous cycle. Journal of Reproduction & Fertility 19, 547-549.

Mattner, P.E. & Thorburn, G.D. (1970). Chronic diversion of utero-ovarian venous blood into the anterior mammary vein of the ewe. Journal of Reproduction & Fertility 21, 370-371.

Mauléon, P. & Denamur, R. (1966). Luteal maintenance in hypophysectomized and hysterectomized sheep. Quoted by Thibault, C. (1966).

McClellan, M.C., Abel, J.H. & Niswender, G.D. (1977). Function of lysosomes during luteal regression in normal cycling and $PGF_2\alpha$ - treated ewes. Biology of Reproduction 16, 499-512.

McCracken, J. (1971). Prostaglandin $F_2\alpha$ and corpus luteum regression Annals of the New York Academy of Sciences 180, 456-469.

Loeb, L. (1916). Further investigations on the cyclic changes in the mammalian ovary. Proceedings of the Society for Experimental Biology and Medicine 13, 162-164.

Leob, L, (1923a). The effect of extirpation of the uterus on the life and function of the corpus luteum in the guinea pig. Proceedings of the Society for Experimental Biology and Medicine 20, 441-443.

Loeb, (1923b). The mechanism of the sexual cycle with special reference to the corpus luteum. American Journal of Anatomy 32, 305-343.

Loeb, L. (1927). The effects of hysterectomy on the system of sex organs and on the periodicity of the sexual cycle in the guinea pig. American Journal of Physiology 83, 202-224.

Louis, T.M., Parry, D.M., Robinson, J.S., Thorburn, G.D. & Challis, J.R.G. (1977). Effects of exogenous progesterone and oestradiol on prostaglandin F and 13-14-Dihydro-15-oxo prostaglandin $F_2\alpha$ concentrations in uteri and plasma of ovariectomized ewes. Journal of Endocrinology 73, 427-439.

Machline, L.J., Takahashi, Y., Horino, M., Hertelendy, F., Gordon, R.S. & Kipnis, D. (1968). Regulation of growth hormone secretion in non-primate species. In: Proceedings of the first International Symposium on Growth Hormone, Eds. A. Pecile & E.E. Muller, Amsterdam, Excerpta Medica Foundation. P. 292-305.

Mapletoft, R.J. & Ginther, O.J. (1975). Adequacy of main uterine vein and the ovarian artery in the local venoarterial pathway for uterine induced luteolysis in ewes. American Journal of Veterinary Research 36, 957-963.

McCracken, J.A. & Baird, D.T. (1969). The study of ovarian function by means of transplantation of the ovary in the ewe. In: The Gonads, K.W. McKerns, ed., Appleton-Century-Crofts, New York, Chapt 7, p 175-209.

McCracken, J.A., Baird, D.T. Carlson, J.C. Goding, J.R. & Barcikowski, B. (1973). The role of prostaglandins in luteal regression. Journal of Reproduction & Fertility Suppl. 18, 133-142.

McCracken, J.A., Baird, D.T. & Goding, J.R. (1971). Factors affecting the secretion of steroids from the transplanted ovary in sheep. Recent Progress in Hormone Research 27, 537-582.

McCracken, J.A., Barcikowski, B., Carlson, J.C., Gréen, K. & Samuelsson, B. (1973). The physiological role of prostaglandin $F_2\alpha$ in corpus luteum regression. Advances in the Biosciences 9, 599-624.

McCracken, J.A. & Caldwell, B.V. (1969). Corpus luteum maintenance in a ewe with one congenitally absent uterine horn. Journal of Reproduction & Fertility 20, 139-141.

McCracken, J.A., Caldwell, B.V., Tillson, S.A., Thorneycroft, I.H. & Scaramuzzi, R.J. (1969). Ovarian steroid secretion and LH levels in sheep after autotransplantation of the ovary, uterus, cervix and vagine to the neck. 2nd Annual Meeting of the Society of Study of Reproduction., Davis, California, p 2.

McCracken, J.A., Carlson, J.C., Glew, M.E., Goding, J.R., Baird, D.T., Green, K. & Samuelsson, B. (1972). Prostaglandin $F_2\alpha$ identified as luteolytic hormone in sheep. Nature, New Biology 238, 129-134.

McCracken, J.A. & Einter-Jensen, N (1979). The counter-current transfer of progesterone in the ovarian vascular pedicle. Fifth International Congress of Endocrinology, Hamburg, Abst. 775, p 320.

McCracken, J.A., Glew, M.E. & Levy, L.K. (1970). Regulation of corpus luteum function by gonadotropins and related compounds. Advances in the Biosciences 4, 377-397.

McCracken, J.A., Glew, M.E. & Scaramuzzi, R.J. (1970). Corpus luteum regression induced by prostaglandin $F_2\alpha$. Journal of Clinical Endocrinology and Metabolism 30, 544-546.

McCracken, J.A., Uno, A., Goding, J.R., Ichikawa, Y. & Baird, D.T. (1969). The in vivo effects of sheep pituitary gonadotrophins on the secretion of steroids by autotransplanted ovary of the ewe. Journal of Endocrinology 45, 425-440.

McKenzie, F.F. & Terrill, C.E. (1937). Estrus, ovulation and related phenomena in the ewe. Missouri Agricultural Experiment Station, Research Bulletin 264, 1-88.

McNatty, K.P., Revfeim, K.J.A. & Young, A. (1973). Peripheral plasma progesterone concentrations in sheep during the oestrous cycle. Journal of Endocrinology 58, 219-225.

Meckley, P.E. & Ginther, O.J. (1969). Lymphatic drainage of uterus and ovaries in the ewe. Journal of Animal Science 29, 195.

Melampy, R.M. & Anderson, L.L. (1968). Role of the uterus in corpus luteum function. Journal of Animal Science 27, Suppl. 1, 77-96.

Mitchell, S., Poyser, N.L. & Wilson, N.H. (1977). Effect of P-Bromophenacyl bromide, an inhibitor of phospholipase A_2, on arachidonic acid release and prostaglandins synthesis by the guinea-pig uterus in vitro. British Journal of Pharmacology 59, 107-114.

Moor, R.M. (1968). Effect of embryo on corpus luteum function. Journal of Animal Science 27, Suppl. 1, 97-118.

Moor, R.M., Booth, W.D. & Rowson, L.E.A. (1966). Effect of hysterectomy on the life-span of the corpora lutea induced artificially in progesterone-treated ewes. Journal of Reproduction & Fertility 12, 385-387.

Moor, R.M., & Rowson, L.E.A. (1964). Influence of the embryo and uterus on luteal function in sheep. Nature, London 201, 522-523.

Moor, R.M. & Rowson, L.E.A. (1966a). Local uterine mechanisms affecting luteal function in the sheep. Journal of Reproduction & Fertility 11, 307-310.

Moor, R.M. & Rowson, L.E.A. (1966b). Local maintenance of the corpus luteum in sheep with embryos transferred to various isolated portions of the uterus. Journal of Reproduction & Fertility 12, 539-550.

Moor, R.M. & Rowson, L.E.A. (1966c). The corpus luteum of the sheep: Functional relationship between the embryo and the corpus luteum. Journal of Endocrinology 34, 233-239.

Moor, R.M.& Rowson, L.E.A. (1966d). The corpus luteum of the sheep: Effect of the removal of the embryos on luteal function. Journal of Endocrinology 34, 497-502.

Moor, R.M. & Rowson, L.E.A. (1970). Quoted by McCracken, J.A., Baird, D.T. & Goding, J.R. (1971).

Moore, N.W., Barrett, S., Brown, J.B., Schindler, I., Smith, M.A. & Smyth, B. (1969). Oestrogen and progesterone content of ovarian vein blood of the ewe during the oestrous cycle. Journal of Endocrinology 44, 55-62.

Moore, N.W., Brown, J.B. & Smyth, B. (1965). In Mattner & Thorburn (1969).

Moore, W.W. & Nalbandov, A.V. (1953). Neurogenic effects of uterine distention on the estrous cycle of the ewe. Endocrinology 53, 1-11.

Morris, B. & Sass, M.B. (1966). The formation of lymph in the ovary. Proceedings of the Royal Society, London, Series B. 164, 577-591.

Mueller, G.C., Herranen, A.M. & Jervell, K.F. (1958). Studies on the mechanism of action of oestrogens. Recent Progress in Hormone Research 14, 95-139.

Nalbandov, A.V. & Cook, B. (1968). Reproduction. Annual Review of Physiology 30, 245-278.

Nalbandov, A.V., Moore, W.W. & Norton, H.W. (1955). Further studies on the neurogenic control of the oestrous cycle by uterine distention. Endocrinology 56, 225-231.

Nalbandov, A.V. & St. Clair, L.E. (1958). Relation of the nervous system to implantation. In: Reproduction and Infertility, IIIrd Symposium, Ed. F.X. Gassner, Pergamon Press, p 83-87.

Naylor, B. & Poyser, N.L. (1975). Effects of oestradiol and progesterone on the in vitro production of prostaglandin $F_2\alpha$ by the guinea-pig uterus. British Journal of Pharmacology 55, 229-232.

Neher, G.M. & Zarrow, M.X. (1954). Concentration of progestin in the serum of the non-pregnant, pregnant and post-partum ewe. Journal of Endocrinology 11, 323.330.

Nett, T.M., McClellan, M.C. & Niswender, G.D. (1976). Effects of prostaglandins on the ovine corpus luteum: Blood flow, secretion of progesterone and morphology. Biology of Reproduction 15, 66-78.

Niswender, G.D. (1968). Discussion. Journal of Animal Science 27, Suppl. 1. 133-138.

Niswender, G.D. (1972). The effect of ergocornine on reproduction in sheep. Biology of Reproduction 7, 138-139.

Niswender, G.D. Diekman, M.A., Nett, T.M. & Akbar, A.M. (1973). Relative blood flow to the ovaries of cycling and pregnant ewes. Biology of Reproduction 9, 87.

Niswender, G.D., Dziuk, P.J., Graber, J. & Kaltenbach, C.C. (1970). Function of the corpora luteum in the ewe following relocation of the uterus or embryo. Journal of Animal Science 30, 935-940.

Niswender, G.D., Kaltenbach, C.C. & Dziuk, P.J. (1967). Autotransplantation of the uterus in sheep. Journal of Animal Science 26, 948-949.

Niswender, G.D., Reichert, L.E., Midgley, A.R., & Nalbandov, A.V. (1969). Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84, 1166-1173.

Niswender, G.D., Reimers, T.J., Diekman, M.A. & Nett, T.M. (1976). Blood flow: A mediator of ovarian function. Biology of Reproduction 14, 64-81.

Norman, R.L., Eleftheriou, B.E., Spies, H.G. & Hoppe, P. (1968). Free plasma estrogens in the ewe during estrous cycle. Steroids 11, 667-671.

Obst, J.M., Seamark, R.F. & Brown, J.M. (1971). Application for a competitive protein binding assay for oestrogens to the study of ovarian function in sheep. Journal of Reproduction & Fertility 24, 140.

Olourfa, M.M., Inskeep, E.K., Pope, A.L. & Casida, L.E. (1961). Effect of uterine distention on the estrous cycle of the mature ewe. Journal of Animal Science 20,975-976

O'Mary, C.C., Pope, A.L. & Casida, L.E. (1950). The use of progesterone in the synchronization of the estrual periods in a group of ewes and the effect on their subsequent lambing records. Journal of Animal Science 9, 499-503.

O'Shea, J.D., Nightingale, M.G. & Chamley, W.A. (1977). Changes in small blood vessels during cyclical luteal regression in sheep. Biology of Reproduction 17, 162-177.

Pant, H.C.,Hopkinson, C.R.M. & Fitzpatrick, R.J. (1977). Concentration of oestradiol, progesterone, luteinizing hormone and follicle-stimulating hormone in the jugular venous plasma of ewes during the oestrous cycle. Journal of Endocrinology 73, 247-255.

Pelletier, J. & Signoret, J-P. (1969). Contrôle de la décharge de LH dans le sang par la progesterone et le benzoate d'oestradiol chez la brebis castrée. Compte rendu hebdomadaire des séances de l'Academie des Sciences 269, 2592-2598. (Série D).

Pexton, J.E., Ford, S.P., Wilson, L., Butcher, R.L. & Inskeep, E.K. (1973). Prostaglandins $F_2\alpha$ in IUD-treated ewes. Journal of Animal Science 37, 324.

Pexton, J.E., Ford, S.P., Wilson, L.,Butcher, R.L. & Inskeep, E.K. (1975). Prostaglandins F in uterine tissue and venous plasma of ewes with intra-uterine devices. Journal of Animal Science 41, 155-153.

Pharriss, B.B., Cornette, J.C. & Gutknecht, G.D. (1970). Vascular control of luteal steroidogenesis. Journal of Reproduction & Fertility, Suppl. 10, 97-103.

Pharriss, B.B. & Wyngarten, L.J. (1969). The effect of prostaglandin $F_{1\alpha}$ on the progestogen content of ovaries from pseuodpregnant rats. Proceedings of the Society for Experimental Biology and Medicine 130, 92-94.

Piper, E.L. & Foote, W.C. (1965). Luteotrophic effect of estradiol in the ewe. Journal of Animal Science 24, 927-928.

Piper, E.L. & Foote, W.C. (1967). Some effects of estradiol on pituitary-ovarian function. Journal of Animal Science 26, 949.

Piper, E.L. & Foote, W.C. (1968). Ovulation and corpus luteum maintenance in ewes treated with 17β-oestradiol. Journal of Reproduction & Fertility 16, 253-259.

Piper, E.L. & Foote, W.C. (1970). The effect of 17β-estradiol on corpus luteum function in sheep. Biology of Reproduction 2, 48-52.

Piper, P. & Vane, J,(1971). The release of prostaglandins from lung and other tissues. Annals of the New York Academy of Sciences 180, 363-385.

Piper, P.J., Vane, J.R. & Wyllie, J.H. (1970). Inactiviation of prostaglandin by the lungs. Nature, London 225, 600-604.

Plotka, E.D. & Erb, R.E. (1967). Levels of progesterone in peripheral blood plasma during the estrous cycle of the ewe. Journal of Animal Science 26, 1363-1365.

Plotka, E.D., Erb, R.E. & Harrington, R.B. (1970). Female sex steroid relationship during the estrous cycle of the ewe. Journal of Animal Science 30, 412-419.

Powell, W.S., Hammerström, S., Samuelsson, B (1974). Prostaglandin $F_2\alpha$ receptor in ovine corpora lutea. Europian Journal of Biochemistry 41, 103-107.

Powell, W.S., Hammarström, S., Samuelsson, B. & Sjöberg, B. (1974). Prostaglandin $F_2\alpha$ receptor in human corpora lutea. Lancet I, 1120.

Poyser, N.L. (1971). Release of prostaglandins from the uterus, Ph.D Thesis, University of Edinburgh.

Poyser, N.L. (1972). Production of prostaglandins by the guinea-pig uterus. Journal of Endocrinology 54, 147-159.

Poyser, N.L. (1973). The physiology of prostaglandins. In: Clinics in Endocrinology and Metabolism, 2 (No 3). Reproductive Endocrinology and World Population (ed. Loraine, J.A.), p 393-410, London: Sunders and Co.

Poyser, N.L. (1974a). The significance of prostaglandin production by the uterus. Polish Journal of Pharmacology and Pharmacy 26, 189-203.

Poyser, N.L. (1974b). Some aspects of prostaglandins in reproduction. Biochemical Society Transactions 2, 1196-1200.

Poyser, N.L. (1976). Prostaglandin $F_2\alpha$ in the uterine luteolytic hormone in the guinea-pig: The evidence reviewed. Advances in Prostaglandin and Thormboxane Research, Vol. 2., ed. B. Samuelsson & R. Paoletti, Raven Press, New York.

Poyser, N.L. (1977). Physiological roles of prostaglandins in reproduction. In: Prostaglandin and Thormboxanes, ed. F. Berti, B. Samuelsson & G.P. Velo, Plenum Publishing Corporation, New York, p 205-219.

Poyser, N.L. (1978a). Hormonal control of prostaglandin synthesis in reproductive processes. Polish Journal of Pharmacology and Pharmacy 30, 171-181.

Poyser, N.L. (1978b). Involvement of prostaglandins in the female reproductive cycle, Biochemical Society Transactions, 575th Meeting, Glasgow, p 718-722.

Poyser, N.L. (1979). Effect of actinomycin D on uterine prostaglandin production and oestrous cycle length in guinea-pigs. Journal of Reproduction & Fertility 56, 559-565.

Poyser, N.L. & Horton, E.W. (1975). Plasma progesterone levels in guinea-pigs actively immunized against prostaglandin $F_2\alpha$, hysterectomized or treated with intra-uterine indomethacin. Journal of Endocrinology 67, 81-88.

Poyser, N.L. & Maule Walker, F.M. (1979). Antiluteolytic effect of the embryo. In: Maternal Recognition of Pregnancy, Ciba Foundation Series 64, Excerpta Medica, p 261-292.

Pratt, B.R., Butcher, R.L. & Inskeep, E.K. (1975). Intra-follicular $PGF_2\alpha$ on day 13 in bred and non-bred ewes. Journal of Animal Science 41, 374.

Quinlan, J. & Maré, G.S. (1931). The physiological changes in the ovary of the Merino sheep in South Africa and their practical application in breeding. 17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, Sec. VII. p 661-703, Onderstepoort, Pretoria.

Quirke, J.F. & Gosling, J.P. (1976). Progesterone levels throughout the oestrous cycle in three ewe breeds with different ovulation rates. 8th International Congress on Animal Production & Artificial Insemination 3, Krakow, p 180-183.

Quirke, J.F., Hanarahan, J.P. & Gosling, J.P. (1979). Plasma progesterone levels throughout the oestrous cycle and release of LH at oestrus in sheep with different ovulation rates. Journal of Reproduction & Fertility 55, 37-44.

Radford, H.M., Wallace, A.L. & Wheatley, I.S. (1970). LH release, ovulation and oestrus following the treatment of anoestrous ewes with ovarian steroids. Journal of Reproduction & Fertility 21, 371-372.

Radford, H.M., Wheatley, I.S. & Wallace, A.L. (1969). The effects of oestradiol benzoate and progesterone on secretion of luteinizing hormone in the ovariectomized ewe. Journal of Endocrinology 44, 135-136.

Rao, Ch.V. (1974). Receptors for prostaglandins & gonadotropins in the cell membrane of bovine corpus luteum. Prostaglandins 4, 567-576.

Rao, Ch.V., Estergreen, V.L., Carman, F.R. & Moss, G.E. (1979). Receptors for gonadotrophin and prostaglandin $F_2\alpha$ in bovine corpora lutea of early, mid and late luteal phase. Acta Endocrinolgica 91, 529-537.

Reeves, J.J., Arimura, A.& Schally, A.V. (1970). Serum levels of prolactin and luteinizing hormone (LH) in the ewe at various stages of the estrous cycle. Proceedings of the Society for Experimental Biology and Medicine 134, 938-942.

Restall, B.J., Hearnshaw, H.R., Gleeson, A.R. & Thorburn, G.D. (1973). Observations on the luteolytic action of prostaglandin $F_2\alpha$ in the ewe. Journal of Reproduction & Fertility 32, 325-326.

Roberts, J.S., Barcikowski, B., Wilson, L., Skarness, R.C. & McCracken, J.A. (1975). Hormonal and related factors affecting the release of prostaglandin $F_2\alpha$ from the uterus. Journal of Steroid Biochemistry 6, 1091-1097.

Roberts, J.S., Carlson, J.C. & McCracken, J.A. (1976). Prostaglandin $F_2\alpha$ production by the brain and its role in LH secretion. Advances in Prostaglandin and Thromboxane Research, Ed. Samuelsson, B. Paoletti, R., Raven Press, New York, Vol. 2 p 609-619.

Roberts, J.S. & McCracken, J.A. (1976). Does prostaglandin $F_2\alpha$ released from uterus by oxytocin mediate oxytocin action of oxytocin. Biology of Reproduction 15, 457-463.

Roberts, J.S., McCracken, J.A., Gavagan, J.E. & Soloff, M.S. (1976). Oxytocin-stimulated release of prostaglandin $F_2\alpha$ from ovine endometrium in vitro: correlation with estrous cycle and oxytocin-receptor binding. Journal of Endocrinology 99, 1107-1114.

Robertson, H.A. & Sarda, I.R. (1971). A very early pregnancy test for animals: its application to the cow, ewe and sow. Journal of Endocrinology 49, 407-419.

Rothchild, I.(1966). The nature of the luteotrophic process. Journal of Reproduction & Fertility, Suppl. 1. 49-61.

Rowson, L.E.A. & Moor, R.M. (1964). Effect of partial hysterectomy on the length of the dioestrous interval in sheep. Fifth International Congress on Animal Reproduction & Artificial Insemination 2, Trento, p 394-398.

Rowson, L.E.A., Moor, R.M., Hay, M.F. & Caldwell, B.V. (1969). Quoted by Anderson, L.L, Bland, K.P. & Melampy, R.M. (1969).

Salamonsen, L.A., Jonas, H.A., Burger, H.G., Buckmaster, J.M., Chamley, W.A., Cumming, I.A., Findlay, J.K. & Goding, J.R. (1973). A heterologous radioimmunoassay for follicle-stimulating hormone: application to measurement of FSH in the ovine estrous cycle and in several other species including man. Endocrinology 93, 610-618.

Sarda, I.R., Robertson, H.A. & Smeaton, T.C. (1973). Sequential changes in plasma progesterone levels in the ewe during the oestrous cycle and during pregnancy in intact and ovariectomized ewes. Canadian Journal of Animal Science 53, 25-34.

Savard, K., Marsh, J.M. & Rice, B.F. (1965). Gonadotropins and ovarian steroidogenesis. Recent progress in Hormone Research 21. 285-365.

Scaramuzzi, R.J., Baird, D.T., Wheeler, A.G. & Land, R.B. (1973). The oestrous cycle of the ewe following active immunization against prostaglandin $F_2\alpha$. Acta endocrinolgica, Suppl. 177, 318.

Scaramuzzi, R.J., Boyle, H.P., Wheeler, A.G., Land, R.B. & Baird, D.T. (1974). Preliminary studies on the secretion of prostaglandin F from the autotransplanted uterus of the ewe after exogenous progesterone and oestradiol. Journal of Endocrinology 61, xxxv.

Scaramuzzi, R.J., Caldwell, B.V. & Moor, R.M. (1970). Radioimmunoassay of LH and estrogen during the estrous cycle of the ewe. Biology of Reproduction 3, 110-119.

Scaramuzzi, R.J., Tillson, S.A., Thorneycroft, I.H. & Caldwell, B.V. (1971). Action of exogenous progesterone and estrogen on behavioural estrus and luteinizing hormone levels in the ovariectomized ewe. Endocrinology 88, 1184-1189.

Schomberg, D.W. (1967). A demonstration *in vitro* of luteolytic activity in pig uterine flushings. Journal of Endocrinology 38, 359-360.

Schomberg, D.W. (1969a). The concept of a uterine luteolytic hormone. In: The Gonads. K.W. McKern ed., Appleton-Century-Crofts, New York, Chapt. 15. p 383-414.

Schomberg, D.W. (1969b). Effects of pig uterine flushings on ovarian tissues *in vitro* and *in vivo*. Proceedings of the III rd International Congress of Endocrinology, Mexico, 1968. Excerpta Medica International Congress Series 189, 916-920.

Sharma, S.C. & Fitzpatrick, R.J. (1974). Effects of oestradiol 17β and oxytocin treatment on prostaglandin $F_2\alpha$ alpha release in the anoestrous ewe. Prostaglandins 6, 97-105.

Short, R.V. (1958). Progesterone in blood, 1. The chemical determination of progesterone in peripheral blood. Journal of Endocrinology 16, 415-425.

Short, R.V. (1960). Appendix to paper entitled: Egg transfer in sheep. Factors affecting the survival of transferred eggs. By N.W. Moore & L.E.A. Rowson, Journal of Reproduction & Fertility 1, 332-349.

Short, R.V. (1961). Progesterone. In: Hormones in blood, Eds. C.H. Gray & A.L. Bacharach, New York, Academic Press, p 379.

Short, R.V. (1969). Implantation and maternal recognition of pregnanacy. In: Ciba Foundation Symposium, Foetal Autonomy, Eds. G.E.W. Wolstenholme & M. O'Connor, Churchill Ltd., London. p 2-31.

Short, R.V., MacDonald, M.F. & Rowson, L.E.A. (1963). Steroids in the ovarian venous blood of ewes before and after gonadotrophic stimulation. Journal of Endocrinology 26, 155-169.

Skarnes, R.C. & Harper, M.J.K. (1972). Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. Prostaglandins 1, 191-203.

Smeaton, T.C. & Robertson, H.A. (1971). Studies on growth and atresia of graafian follicles in the ovary of the sheep. Journal of Reproduction & Fertility 25, 243-252.

Smith, J.F. & Robinson, T.J. (1969). Luteal function in the Merino ewe and the effect of exogenous progestagen. Journal of Endocrinology 44, 79-89.

Smith, W.L., Husling, R.L. & Fogwell, R.L. (1979). Relationship of the prostaglandin-forming cyclo-oxygenase to luteolysis. Pre-circulated abstracts - International Conference on Prostaglandins. Washington. p 108.

Soloff, M.S. (1976). Oxytocin receptors in rat oviduct. Biochemical and Biophysical Research Communication 66, 671-677.

Spilman, C.H. & Duby, R.T. (1972). Prostaglandin mediated luteolytic effect of an intrauterine device in sheep. Prostaglandins 2, 159-168.

Stabenfeldt, G.H., Holt, J.A. & Ewing, L.L. (1969). Peripheral plasma progesterone levels during the ovine estrous cycle. Endocrinology 85, 11-15.

Stacy, B.D. & Gemmell, R.T. (1976a). Morphological and functional changes in the corpus luteum after restriction of ovarian blood flow. Journal of Reproduction & Fertility 46, 526.

Stacy, B.D., & Gemmell, R.T. (1976b). Induction of regression in the corpus luteum by ICI 80, 996, an analogue or prostaglandin $F_2\alpha$ Journal of Reproduction & Fertility 48, 415-416.

Stacy, B.D., Gemmell, R.T. & Thorburn, G.D. (1975). Morphology of the corpus luteum during normal and prostaglandin-induced luteolysis in sheep. Journal of Reproduction & Fertility 43, 402-403.

Stacy, B.D., Gemmell, R.T. & Thoburn, G.D. (1976). Morphology of the corpus luteum in the sheep during the regression induced by prostaglandin $F_2\alpha$. Biology of Reproduction 14, 280-291.

Stormshak, F. & Hawk, H.W. (1966). Effect of intrauterine spirals and HCG on the corpus luteum of ewe. Journal of Animal Science 25, 931.

Stormshak, F., Inskeep, E.K., Lynn, J.E., Pope, A.L. & Casida, L.E. (1963). Progesterone levels in the corpora lutea and ovarianeffluent blood of the ewe. Journal of Animal Science 22, 1021-1026.

Stormshak, F. & Kelley, H.E. (1967). Action of ovine endometrium on luteal steroidogenesis. Journal of Animal Science 26, 952.

Stormshak, F, Kelley, H.E. & Hawk, H.W. (1969). Suppression of ovine luteal function by 17β-estradiol. Journal of Animal Science 29, 476-478.

Stormshak, F., Lehman, R.P. Hawk, H.W. (1967). Effect of intrauterine plastic spirals and HCG on the corpus luteum of the ewe. Journal of Reproduction & Fertility 14, 373-378.

Strakosch, T., Hecker, J.F., & Wodzicka-Tomaszewska, M. (1973). The effects of intra-uterine devices (IUDs) on ovarian function in sheep, with particular reference to the luteal function. Journal of Reproduction & Fertility 32, 324.

Thibault, C. (1966). Luteal maintenance in hypophysectomized and hysterectomized sheep. Journal of Reproduction & Fertility, Suppl. 1, 63-65.

Thorburn, G.D., Bassett, J.M. & Smith, I.D. (1969). Progesterone concentration in the peripheral plasma of sheep during the oestrous cycle. Journal of Endocrinology 45, 459-469.

Thorburn, G.D., Cox, R.I., Currie, W.B., Restall, B.J. & Schneider, W. (1972). Prostaglandin F concentration in the utero-ovarian venous plasma of the ewe during the oestrous cycle. Journal of Endocrinology 53, 325-326.

Thorburn, G.D., Cox, R.I., Currie , W.B., Restall, B.J. & Schneider, W. (1973). Prostaglandin F and progesterone concentrations in the utero-ovarian venous plasma of the ewe during the oestrous cycle and early pregnanacy. Journal of Reproduction & Fertility. Suppl. 18, 151-158.

Thorburn, G.D.& Hales, J.R.S. (1972). Selective reduction in blood flow to the ovine corpus luteum after infusion of $PGF_{2\alpha}$ into a uterine vein. Proceedings of the Australian Physiological and Pharmacological Society 3, 145.

Thorburn, G.D. & Mattner, P.E. (1969). A method for collecting utero-ovarian venous blood in conscious ewe for assessment of ovarian and uterine steroid production. Australian Journal of Experimental Biology and Medical Science 47, 32p.

Thorburn, G.D. & Mattner, P.E. (1971). Anastomosis of the utero-ovarian and anterior mammary veins for collection of utero-ovarian venous blood: Progesterone secretion rates in cyclic ewes. Journal of Endocrinology 50, 307-320.

Thorburn, G.D. & Nicol, D.H. (1971). Regression of the ovine corpus luteum after infusion of prostaglandin $F_2\alpha$ into the ovarian artery and uterine vein. Journal of Endocrinology 51, 785-786.

Thwaites, C.J. (1971). Exogenous progesterone and oestrous cycle length in the ewe. Journal of Agricultural Science 77, 147-149.

Thwaites, C.J. & Edey, T.N. (1970). Histology of the corpus luteum in the ewe: changes during the oestrous cycle and early pregnancy, and in response to some experimental treatments. American Journal of Anatomy 129, 439-448.

Umo, I. (1975). Effect of prostaglandin $F_2\alpha$ on the ultrastructure and function of sheep corpora lutea. Journal of Reproduction & Fertility 43, 287-292.

Utiger, R.D., Parker, M.L. & Daughaday, W.H. (1962). Studies on human growth hormone. I. A radio-immunoassay for human growth hormone. Journal of Clinical Investigation 41, 254-261.

Wallace, A.L.C. & Bassett, J.M. (1970). Plasma growth hormone concentrations in sheep measured by radioimmunoassay. Journal of Endocrinology 47, 21-36.

Walsh, S.W., Yutrzenka, G.J. & Davies, J.S. (1979). Local steroid concentrating mechanism in the reproductive vasculature of the ewe. Biology of Reproduction 20 1167-1171.

Warbritton, V. (1934). The cytology of the corpora lutea of the ewe. Journal of Morphology 56, 181-202.

Warren, J.E., Hawk, H.W. & Bolt, D.J. (1973). Evidence for prog--estational priming of estradiol-induced luteal regression in the ewe. Biology of Reproduction 8, 435-440.

Wheeler, A.G. & Land, R.B. (1977). Seasonal variance in oestrus and ovarian activity of Finnish Landrace, Tasmanian Merino and Scottish Blackface ewes. Animal Production 24, 363-376.

Wilson, L.,Butcher, R.L. & Inskeep, E.K. (1972). Prostaglandins $F_{2\alpha}$ in the uterus of ewes during early pregnanacy. Prostaglandins 1, 479-482.

Wilson, L., Butcher, R.L., Cenedella, R.J. & Inskeep, E.K. (1972). Effects of progesterone on endometrial prostaglandins in sheep. Prostaglandins 1, 183-190.

Wilson, L., Cenedella, R.J., Butcher, R.L. & Inskeep, E.K. (1971). Progesterone treatment on ovine endometrial prostaglandins. Journal of Animal Science 33, 273.

Wilson, L., Cenedella, R.J., Butcher, R.L. & Inskeep, E.K. (1972). Levels of prostaglandins in the uterine endometrium during the ovine estrous cycle. Journal of Animal Science 34, 93-99.

Wiltbank, J.N. & Casida, L.E. (1956). Alteration of ovarian activity by hysterectomy. Journal of Animal Science 15, 134-140.

Wlodawer, P., Kindhahl, H. & Hamberg, M. (1976). Biosynthesis of prostaglandin $F_{2\alpha}$ from arachidonic acid and prostaglandin end_operoxides in the uterus. Biochemica et Biophysica Acta 431, 603-614.

Wodzicka-Tomaszewska, M., Hecker, J.F., & Bary, A.R., (1973). Effect of day of insertion of intra-uterine devices (IUDs) on luteal function in sheep. Journal of Reproduction & Fertility 32, 323-324.

Woody, C.O. (1968). Discussion, Journal of Animal Science 27, Suppl. 1, 130.

Woody, C.O., First, N.L. & Pope, A.L. (1967). Effect of exogenous progesterone on estrous cycle length. Journal of Animal Science 26, 139-141.

Woody, C.O., Ginther, O.J. & Pope, A.L. (1966a). Effects of hysterectomy and exogenous progesterone on the corpus luteum of the ewe. Journal of Animal Science 25, 933.

Woody, C.O., Ginther, O.J. & Pope, A.L. (1966b). Effects of exogenous progesterone on corpora lutea induced in anestrous ewes. Journal of Animal Science 25, 1263.

Woody, C.O., Ginther, O.J. & Pope, A.L. (1967). Effects of exogenous progesterone on corpora lutea induced in anestrous ewes. Journal of Animal Science 26, 1113-1115.

Woody, C.O., Ginther, O.J. & Pope, A.L. (1968). Effects of exogenous progesterone and hysterectomy on corpora lutea in ewes. Journal of Animal Science 27, 1383-1386.

Woody, C.O., Ginther, O.J. & Pope, A.L. (1969). Unilateral effect of intra-uterine injection of alcoholic solutions on the corpus luteum of the ewe. Journal of Animal Science 28, 63-65.

Yuthasastrakosol, P., Palmer, W.M. & Howland, B.E. (1975). Luteinizing hormone, oestrogen and progesterone levels in peripheral serum of anoestrous and cyclic ewes as determined by radio immunoassay. Journal of Reproduction & Fertility 43, 57-65.

Yuthasastrakosol, P., Palmer, W.M. & Howland, B.E. (1977). Release of LH in anoestrous and cyclic ewes. Journal of Reproduction & Fertility 50, 319-321.

Zhordania, I.F. & Gotsiridze, O.A. (1963). Autotransplantation of the uterus into the omentum. International Journal of Fertility 8, 849-857.

Zimbelman, R.G., Pope, A.L. & Casida, L.E. (1959). Effect of exogenous progesterone on the corpus luteum of the bred ewe. Journal of Animal Science 18, 1327-1332.

APPENDIX 1:

Publications resulted from this work:

Alwachi, S.N., Bland, K.P. & Poyser, N.L. (1979). Uterine prostaglandin $F_2\alpha$ & E_2 production and content during the second half of the oestrous cycle of the sheep-possible local control of the uterus by the ovary. Prostaglandins & Medicine 3 (1), 23-32

APPENDIX II:

Instructions for Nakayama's Instrument for Small Vessel Anastomosis.

APPENDIX I

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UTERINE PROSTAGLANDIN $F_{2\alpha}$ & E_2 PRODUCTION AND CONTENT DURING THE SECOND HALF OF THE OESTROUS CYCLE OF THE SHEEP - POSSIBLE LOCAL CONTROL OF THE UTERUS BY THE OVARY

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ABSTRACT

The amount of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) in the endometrial tissue of sheep and the ability of this tissue to synthesize these prostaglandins have been monitored daily during the latter part of 12 oestrous cycles in seven ewes. Plasma concentrations of $PGF_{2\alpha}$ and progesterone were measured simultaneously during ten of these cycles. No difference could be found between the content of or the synthesizing ability for $PGF_{2\alpha}$ or PGE_2 of caruncular and non-caruncular endometrial tissue, although the myometrium contained and produced significantly less $PGF_{2\alpha}$ than either of these tissues.

There was no relationship between $PGF_{2\alpha}$ content, secretion and synthesizing ability of endometrial tissue although changes in the uterine content of PGE_2 and $PGF_{2\alpha}$ were directly related. Changes in the ability of the uterine endometrium to synthesize $PGF_{2\alpha}$ varied with the presence or absence of the neighbouring ovary. When the adjacent ovary was present there was a statistically significant increase in the ability of the endometrium to synthesize $PGF_{2\alpha}$ 3 and 2 days before the onset of oestrus. In the absence of the ovary this increase did not occur. The data suggests that the ovary has a local controlling influence over the ability of the endometrium to synthesize $PGF_{2\alpha}$.

INTRODUCTION

The role of the uterus in the control of the lifespan of the ovine corpus luteum and hence the length of the oestrous cycle is now well established (Horton & Poyser, 1976) : The local pathway by which the luteolytic hormone prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) gets from the uterus to the ovary has also been carefully researched (see Goding, 1974). The present study is concerned with investigating the mechanisms which control the production of $PGF_{2\alpha}$ by the ovine uterus. Simultaneous measurements of the uterine content of $PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2), of the ability of the endometrial tissue to synthesize prostaglandins and of the concentration of $PGF_{2\alpha}$ in the uterine vein have been made. The relationship between these values should indicate that area of prostaglandin synthesis at which extra-uterine factors act to cause the increase in the release of $PGF_{2\alpha}$ needed for luteolysis.

MATERIALS AND METHODS

General preparation of animals and anaesthesia

Mature non-pregnant Dorset Horn x Finnish landrace ewes were used. Oestrus was detected in all animals by daily testing with a vasectomized ram. The first day of oestrus was taken as day 1 of the cycle. The ewes were kept in individual pens under natural daylight and fed about 1500 g of hay daily.

Food was withheld for 24 h. before surgery. Anaesthesia was induced either with intravenous injection of 20 ml Saffan (alphaxalone & alphadolone acetate, Glaxo Laboratories Ltd., Greenford, Middlesex) or with nitrous oxide and oxygen mixture. All operations were carried out with full aseptic surgical procedures. A preliminary experiment was undertaken before embarking on the main investigation.

Preliminary Experiment :

From seven ewes duplicate samples of caruncular and non-caruncular endometrium, and of myometrium, were taken by laparotomy during anaesthesia on various days of the oestrous cycle. The content and synthesizing capacity of the different uterine tissues were measured by the same methods as for the main experiment.

Main Experiment :

Two groups of animals were used :-

Group 1 : Four ewes in which anastomosis of one uterine vein to the anterior mammary vein was attempted, with simultaneous removal of the adjacent ovary. The uterine horn on the operated side was then fistulated to the exterior.

Group 2 : Two ewes in which anastomosis was attempted and unilateral ovariectomy was performed as for group 1, but the uterine horn on the unoperated side was fistulated to the exterior. In a further animal fistulation of one uterine horn was performed without previously removing an ovary and attempting a utero-mammary vein anastomosis.

Vessel Anastomosis :

Vessel anastomosis was necessary as numerous attempts at cannulation of the uterine vein in non-pregnant sheep for long-term sampling had proved unreliable. End to end anastomosis of the uterine vein to the anterior mammary vein (vena epigastrica cranialis superficialis) was adapted from the method of Thorburn & Mattner (1971). In the current experiments the utero-ovarian vein and artery were not separated, but instead the adjacent ovary was removed. When healing was complete and the ewe had returned to oestrus at least once, the patency of anastomosis was checked by the retrograde injection of 5 ml of Urografin 76% (Schering Chemicals Ltd., Burgess Hill, Sussex) into the anastomosed utero-mammary vein, and the vessels were visualised by x-radiography. If the anastomosis was patent, samples of uterine venous blood could thus be obtained by simple venipuncture from the anastomosed utero-mammary vein. At postmortem the patency of the anastomosis was rechecked by injection of either Indian ink or coloured gelatin solution.

In three animals (one in group 1 and both those in group 2) the anastomosis was found not to be patent and thus the blood concentrations of the different components in these three animals are equivalent to peripheral levels. In the animal in group 1 the uterine drainage was to the ventral abdominal muscle but not into the anterior mammary vein, while in both animals in group 2 the uterine venous drainage had re-established its pre-operative route.

Fistulation :

After the re-establishment of normal oestrous cycles, the base of the left uterine horn was fistulated to the exterior. The uterus was located through the mid-ventral incision just to the left of the midline, and feed from any adhesions that were present. One uterine horn was ligated at the base and severed. The basal end was then passed through a small hole in the abdominal wall just in front of the udder and sutured in position with single interrupted sutures through all layers. A dressing and antibiotics (Streptopen Q.R. Cerate, Glaxo Veterinary, Greenford, Middlesex) were applied to the fistula to prevent contamination and the dressing was changed daily.

Samples :

Samples were taken daily at approximately 10.00 h. At each sampling two endometrial biopsies (each greater than 20 mg) were obtained, using a home-made stainless-steel biopsy instrument, and about 3 ml of venous blood were taken by venipuncture from the mammary vein. Each endometrial biopsy was described by visual examination, as either caruncle or non-caruncular tissue and weighed. To ascertain the initial prostaglandin content of the endometrial tissue one sample was immediately placed in 5 ml absolute ethyl alcohol and stored at -20°C. This sample was later homogenized in alcohol in which it had been stored and centrifuged. The supernatant was withdrawn and the precipitate washed in 5 ml absolute ethanol, recentrifuged and the washings combined with the original extract. The alcoholic extract was evaporated to dryness and the residue dissolved in 10 ml water. The pH of the aqueous extract was then lowered to 4.5 and the prostaglandins were

extracted as described below and stored in 2 ml ethyl acetate at -20°C until assayed. To obtain an indication of the ability of the endometrial tissue to synthesize $PGF_{2\alpha}$ and PGE_2, albeit under in vitro conditions, the other endometrial tissue sample was homogenized in 15 ml Tyrode's solution with a Fison's glass homogenizer and incubated for 90 min. at 37°C with a constant stream of oxygen bubbling through it. Prostaglandin synthesis was halted after incubation by adjusting the pH to 4.5 with M HCl. The sample was extracted three times with 40 ml redistilled ethyl acetate and the three fractions combined. After washing with distilled water (pH less than 6.0), the combined fraction was evaporated to dryness and the residue redissolved in 5 ml ethyl acetate and stored at -20°C until analysed. Blood samples were centrifuged at 1500g for 10 min, and the plasma removed and stored at -20°C.

Progesterone and $PGF_{2\alpha}$ concentrations in plasma, and $PGF_{2\alpha}$ and PGE_2 in extracts of uterine tissue were measured by radioimmunoassay using methods previously described (Blatchley & Poyser, 1974; Dighe, Emslie, Henderson, Rutherford & Simon, 1975; Poyser & Horton, 1975; Fenwick, Jones, Naylor, Poyser & Wilson, 1977). The one-step extraction procedures that were used give greater than 90% recovery of $PGF_{2\alpha}$, PGE_2 and progesterone. Within assay coefficients of variation were 12.3, 12.1 and 11.9% for progesterone, $PGF_{2\alpha}$ and PGE_2 respectively. The between assay coefficients of variation were 11.2, 10.3 and 13.5% respectively. The limits of detection of the assays were, progesterone, 20pg; $PGF_{2\alpha}$, 40pg; PGE_2, 4 pg. Using the extraction methods outlined the mean variation between duplicate samples was 26% and 25% for $PGF_{2\alpha}$ and PGE_2 content respectively and 23% and 22% for synthesizing ability for $PGF_{2\alpha}$ and PGE_2 respectively.

Experimental Procedure :

The final arrangement in the animals in the two groups is shown in Figure 1 along with the number of cycles monitored.

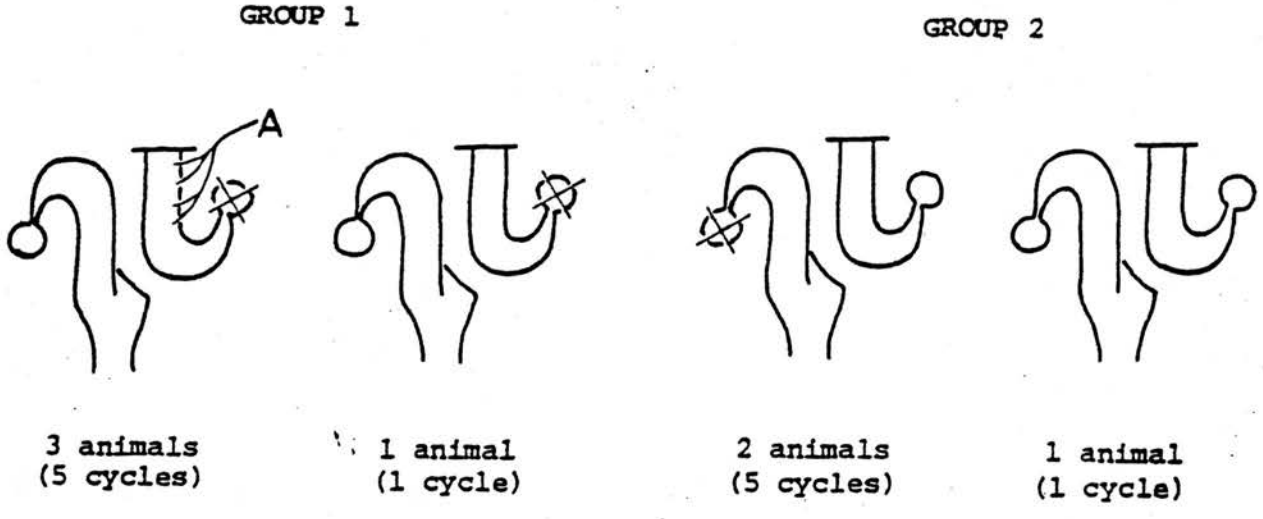

Figure 1 : Diagram of the final arrangement of the genital tract in animals in groups 1 & 2, e.g. In group 1, 3 animals had the left ovary removed and the left uterine vein anastomosed (A) to the mammary vein. The left uterine horn was then fistulated to the exterior.

Thus :

Group 1 : In all 6 cycles no ovary was adjacent to the fistulated uterine horn and the venous drainage of the fistula had been interfered with. In 5 of the cycles a patent utero-mammary anastomosis was present so concentrations of $PGF_{2\alpha}$ and progesterone in the uterine vein could be measured. In the remaining cycle the plasma values are peripheral concentrations.

Group 2 : In all 6 cycles an ovary remained attached to the fistulated uterine horn and the utero-ovarian drainage on that side was unaltered. In all but one cycle no ovary was present on the contralateral side. Plasma concentrations of $PGF_{2\alpha}$ and progesterone represent peripheral values as they were taken from an unanastomosed mammary vein.

Daily samples of uterine tissue were taken during the latter part (day 12 onwards) of a total of 12 oestrous cycles and simultaneous venous blood samples were taken during ten of these cycles. Duplicate tissue samples were taken during one of the cycles investigated in a ewe in group 1. During this cycle duplicate incubations were performed to find whether the availability of arachidonic acid was a limiting factor in tissue prostaglandin synthesis in vitro. Arachidonic acid (20 µg) in 20 µl ethyl alcohol was added to one homogenate while the solvent alone was added to the other homogenate.

Statistical Analysis :

The significance of the differences between various days of the cycle and between the various tissues was checked by using Student's t-test for paired groups. The relationship between the various parameters measured was checked by calculating the correlation coefficients of the regression lines.

RESULTS

Table 1 summarizes the results from the preliminary experiment. Endometrial $PGF_{2\alpha}$ and PGE_2 content were significantly greater ($P < 0.001$ for $PGF_{2\alpha}$ and $P<0.05$ for PGE_2) towards the end of the cycle. There was no difference in the prostaglandin content or synthesizing ability between caruncular and non-caruncular tissue. However, the $PGF_{2\alpha}$ content and synthesizing ability of myometrium was much lower than than for endometrium.

In the main experiment, the visual discrimination between caruncular and non-caruncular endometrium, obtained by biopsy from the fistulated uterine horn, proved easier than anticipated. In no instance was myometrial tissue retrieved by these biopsies, and this was confirmed by our failure to find any damage to the myometrium of the fistulated horn at postmortem examination. Again no difference was found in prostaglandin content and synthesizing ability between caruncular and non-caruncular endometrial tissue, therefore no distinction has been made between the two types of endometrial tissue in assessing the results.

TABLE 1 : The prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) content and synthesizing ability of uterine myometrium and caruncular and non-caruncular endometrium of sheep.

Ewe No.	Day of Oestrous Cycle	Myometrium		Caruncular Endometrium		Non-caruncular Endometrium	
		a $PGF_{2\alpha}$	b PGE_2	c $PGF_{2\alpha}$	d PGE_2	e $PGF_{2\alpha}$	f PGE_2
Tissue content		(ng mg^{-1})					
1	3	0.019	0.011	0.004	0.001	0.004	0.001
2	5	0.004	0.008	0.013	0.009	0.028	0.009
3	5	0.020	0.050	0.060	0.010	0.030	0.008
4	12	0.010	0.006	0.050	0.012	0.10	0.021
5	15	0.030	0.017	0.15	0.022	0.17	0.021
6	15	0.009	0.004	0.17	0.11	0.23	0.15
7	16	0.040	0.006	0.16	0.030	0.23	0.030
Synthesizing Ability (ng mg^{-1} 90 min^{-1})							
1	3	0.050	0.015	0.10	0.021	0.18	0.125
2	5	0.012	low	0.25	0.15	0.45	0.24
3	5	0.075	0.16	0.44	0.16	0.35	0.14
4	12	-	-	0.33	0.18	0.44	0.24
5	15	0.23	0.040	1.62	0.58	1.10	0.84
6	15	0.080	0.040	0.36	0.20	0.48	0.21
7	16	0.15	0.008	0.47	0.16	0.61	0.24

The statistical significance of the differences between $PGF_{2\alpha}$ values in the different uterine tissues and also between PGE_2 values in the different tissues are :

Tissue Content : c $\underline{v}$ a, $P<0.02$; e $\underline{v}$ a, $P<0.02$;
d $\underline{v}$ b, $P<0.4$; f $\underline{v}$ b, $P<0.4$.

Synthesizing Ability : c $\underline{v}$ a, $P<0.05$; e $\underline{v}$ a, $P<0.01$;
d $\underline{v}$ b, $P<0.1$; f $\underline{v}$ b, $P<0.1$.

Figure 2 shows the results obtained from the sheep in groups 1 and 2. There was considerable variation in progesterone concentrations between individual animals resulting in the mean level for group 1 being higher than the mean for group 2. However, this difference is mainly due to one group 1 animal (2 cycles) which had very high plasma progesterone concentrations (mean 25.6 ng/ml). Nevertheless progesterone concentrations in both groups fell normally at the end of the cycle indicating that luteal regression had taken place.

Plasma $PGF_{2\alpha}$ concentrations were higher in the anastomosed utero-mammary vein (group 1) than in the non-anastomosed mammary vein (group 2) as would be expected since uterine venous concentrations were measured in group 1 and peripheral concentrations in group 2. One sheep in group 1 did not have a patent anastomosis and as a result peripheral $PGF_{2\alpha}$ levels, similar to group 2, were obtained; these values have not been included in the group mean.

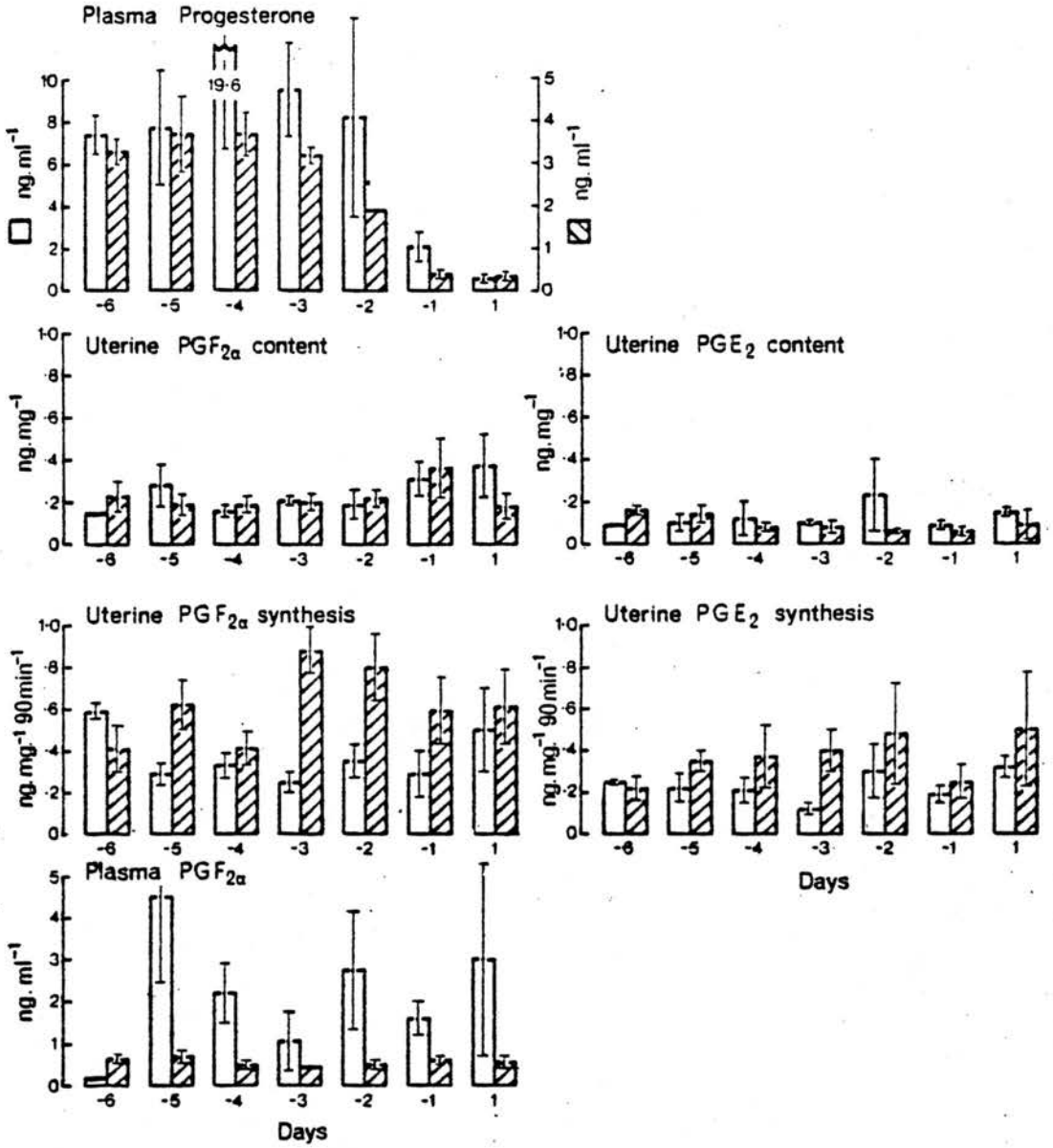

Figure 2 : Mean values (± S.E.) for plasma progesterone, uterine $PGF_{2\alpha}$ & PGE_2 content, uterine $PGF_{2\alpha}$ & PGE_2 synthetic ability and plasma $PGF_{2\alpha}$ in the two groups of sheep. Group 1 (open columns) had no ovary adjacent to the fistula while group 2 (shaded columns) the adjacent ovary was present.

In the sheep in group 1, there were no significant changes in endometrial $PGF_{2\alpha}$ and PGE_2 content or synthesizing ability. Also there was no correlation between endometrial $PGF_{2\alpha}$ content or synthesizing ability and the uterine venous plasma concentration of $PGF_{2\alpha}$. In the sheep in group 2 again there was no significant increase in endometrial $PGF_{2\alpha}$ content, but $PGF_{2\alpha}$ synthesizing ability increased towards the end of the cycle and was significantly higher on days -3 ($P<0.02$) and -2 ($P<0.05$). Between group comparisons also showed that $PGF_{2\alpha}$ synthesizing ability was significantly higher on days -3 ($P<0.01$) and -2 ($P<0.05$) in group 2. There were no significant differences in endometrial PGE_2 synthesizing ability within or between groups. Consequently there was no relationship between the ability

of the endometrium to synthesize $PGF_{2\alpha}$ and PGE_2. Surprisingly, however, there was a direct relationship between the endometrial content of $PGF_{2\alpha}$ and the content of PGE_2, although no relationship existed between the endometrial content of $PGF_{2\alpha}$ and the ability of the endometrium to synthesize $PGF_{2\alpha}$. Changes in the $PGF_{2\alpha}$ content of the endometrium are apparently not related to changes in $PGF_{2\alpha}$ synthesizing ability.

The values obtained on one ewe for duplicate tissue showed that the addition of 20 µg arachidonic acid to one incubate made no significant difference to the amount of $PGF_{2\alpha}$ or PGE_2 synthesized, indicating that the availability of precursor is not a limiting factor in this incubation system.

DISCUSSION

The removal of a biopsy occasionally caused some slight bleeding from the endometrium, but this was temporary and stopped within 30-60 s. Infection in the fistula occurred only on one occasion and the data from this cycle have not been included in the results. The uterine fistula proved to be more resistant to infection than had been expected. All tissue samples were transferred quickly to either alcohol or Tyrode solution. It is unlikely, therefore, that prostaglandin production was appreciably altered by the trauma of removing the samples or by inflammation of the endometrium. The fact that the ewes in group 2 which were unilaterally ovariectomized on the right side and with the left horn fistulated showed cycles of normal length during sampling also supports this.

The values for the content of $PGF_{2\alpha}$ in uterine tissue found in the present experiment are comparable to those found by Wilson, Cenedella, Butcher & Inskeep (1972) and by Lewis and co-workers (1977,1978). However, the absence of consistent differences between $PGF_{2\alpha}$ content of caruncular and non-caruncular tissue appears at first to contradict the findings of Louis, Parry, Robinson, Thorburn & Challis,(1977). They found that the caruncles contained more $PGF_{2\alpha}$ and that they also produced more $PGF_{2\alpha}$ on incubation than did the intercaruncular tissue. However, the intercaruncular tissue used by them consisted of endometrium and myometrium. As there are significantly lower concentrations of $PGF_{2\alpha}$ in the myometrium (see Table 1 and Pexton, Ford, Wilson, Butcher & Inskeep, 1975) their difference was probably due to the inclusion of myometrium in their intercaruncular tissue.

We found no relationship to exist between endometrial $PGF_{2\alpha}$ content, synthesizing ability and secretion in group 1 animals. The animals were sampled on a daily basis and, perhaps, more frequent sampling is required for a relationship to emerge, especially as uterine $PGF_{2\alpha}$ secretion is pulsatile and not continuous. However, in group 1 animals there were no significant increases in uterine $PGF_{2\alpha}$ content or in endometrial $PGF_{2\alpha}$ synthesizing ability over the period studied. This is in contrast to group 2 animal where endometrial $PGF_{2\alpha}$ synthesizing capacity was significantly higher on days -3 and -2. Unfortunately simultaneous measurements of the $PGF_{2\alpha}$ concentration in the uterine venous plasma were not possible in this group.

The increase in $PGF_{2\alpha}$ synthesizing ability of uterus on days -3 in group 2 coincides with the commencement of the fall in plasma progesterone concentrations. These findings hence support the involvement of $PGF_{2\alpha}$ in luteolysis.

The main difference between the animals in groups 1 and 2 was the absence of an ovary adjacent to the fistulated uterine horn in the former group. The fact that the endometrial $PGF_{2\alpha}$ synthesizing ability increased towards the end of the cycle when the adjacent ovary was present (group 2) but not when it was absent (group 1) suggests that the ovary exerts a local control over endometrial $PGF_{2\alpha}$ synthesis in the adjacent uterine horn. Consequently the reason for the lack of any correlation between endometrial $PGF_{2\alpha}$ synthesizing ability and release in the sheep in group 1 may be due to a lack of any local ovarian influence.

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REFERENCES

Blatchley, F.R. & Poyser, N.L. (1974). The effect of oestrogen and progesterone on the release of prostaglandins from the uterus of the ovariectomized guinea-pig. Journal of Reproduction and Fertility, 40, 205-209.

Dighe, K.K., Emslie, H.A., Henderson, L.K., Rutherford, F. & Simon, L.(1975). The development of antisera to prostaglandins E_2 and $F_{2\alpha}$ and their analysis using solid-phase and double antibody radioimmunoassay methods. British Journal of Pharmacology. 55, 503-514.

Fenwick, L., Jones, R.L., Naylor, E.,Poyser, N.L. & Wilson, N.H. (1977). Production of prostaglandins by the pseudopregnant rat uterus, in vitro, and the effect of tamoxifen with the identification of 6-keto-prostaglandin $F_{1\alpha}$ as a major product. British Journal of Pharmacology, 59, 191-199.

Goding, J.R. (1974). The demonstration that $PGF_{2\alpha}$ is the uterine luteolysin in the ewe. Journal of Reproduction and Fertility. 38, 261-271.

Horton, E.W. & Poyser, N.L.(1976). Uterine luteolytic hormone : a physiological role for prostaglandin $F_{2\alpha}$. Physiological Reviews. 56, 595-651.

Lewis, G.S., Jenkins, P.E., Fogwell, R.L. & Inskeep, E.K. (1978). Concentrations of prostaglandins E_2 and $F_{2\alpha}$ and their relationship to luteal function in early pregnant ewes. Journal of Animal Science. 47, 1314-1323.

Lewis, G.S., Wilson, L. Wilks, J.W., Pexton, J.E., Fogwell, R.L., Ford,S.P., Butcher, R.L., Thayne, W.V. & Inskeep, E.K. (1977). $PGF_{2\alpha}$ and its metabolites in uterine and jugular venous plasma and endometrium of ewes during early pregnancy. Journal of Animal Science. 45, 320-327.

Louis, T.M., Parry, D.M., Robinson, J.S., Thorburn, G.D. & Challis, J.R.G. (1977). Effects of exogenous progesterone and oestradiol on prostaglandin F and 13, 14 - dihydro -15- oxo-prostaglandin $F_{2\alpha}$ concentrations in uteri and plasma of ovariectomized ewes. Journal of Endocrinology, 73, 427-439.

Pexton, J.E., Ford, S.P., Wilson, L., Butcher, R.L. & Inskeep, E.K. (1975). Prostaglandin F in uterine tissue and venous plasma of ewes with intrauterine devices. Journal of Animal Science. 41, 144-153.

Poyser, N.L. & Horton, E.W. (1975). Plasma progesterone levels in guinea-pigs actively immunized against prostaglandin $F_{2\alpha}$, hysterectomized or treated with intrauterine indomethacin. Journal of Endocrinology. 67, 81-88.

Thorburn, G.D. & Mattner, P.E. (1971). Anastomosis of the utero-ovarian and anterior mammary veins for collection of utero-ovarian venous blood : progesterone secretion rates in cyclic ewes. Journal of Endocrinology. 50, 307-320.

Wilson, L., Cenedella, R.J., Butcher, R.L. & Inskeep, E.K. (1972). Levels of prostaglandins in the uterine endometrium during the ovine oestrous cycle. Journal of Animal Science. 34, 93-99.

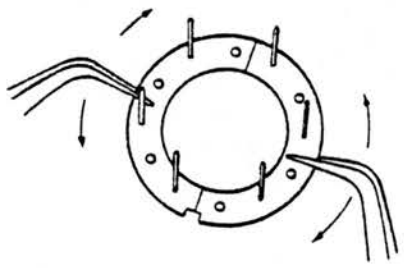
APPENDIX II

Instruction on Nakayama's Instrument for Small Vessel Anastomosis

1) Rings slightly larger than the external diameter of the vessels to be anastomosed are recommended.

2) Before splitting the rings, each ring should be tested to determine if it is properly set in the indentation of the vascular anastomosis clamp (ring-holding clamp) of the corresponding size. (A shallow incision line is made on each ring for easy bisection on use.)

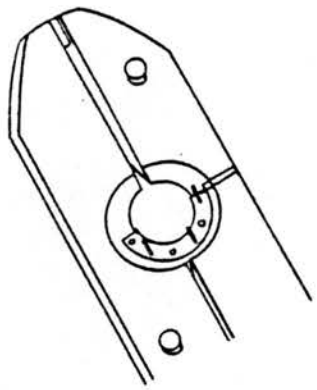
3) Then, the rings are split by a few bending motions with the specially designed forecp (fig. 1.).



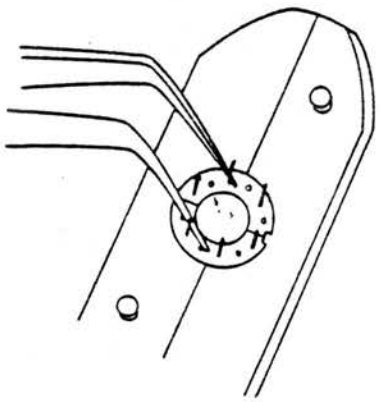
(fig. 1)

4) Separated pieces of each ring should be used as one pair throughout the setting process. Open slightly the vascular anastomosis clamps. One ring half is placed irst in the proximal half of the indentation of the clamp, and the other is placed in the distal half (fig. 2).

It is recommended that the indentation be sustained evenly (horizontally) while setting the halves. The ring is easily cracked away while being manipulated. It is advisable that the setting process be done most gently with our special forcepses on a large aseptic table. After satisfactorily setting the ring, gently close the tips of the vascular anastomosis clamp with applying a forcep over the ring in the indentation to prevent it from falling off (fig. 3.).



(fig. 2)



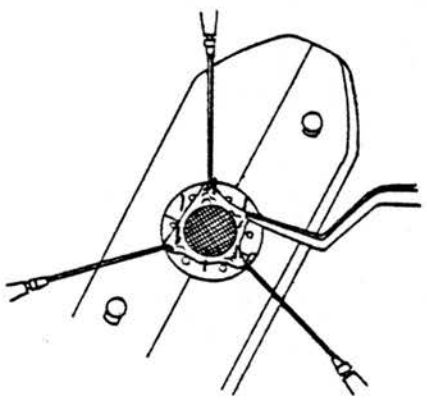
(fig. 3)

If the ring is not properly set, and the two pieces are not on the same exact plane, the setting should be repeated. If after a few attempts the ring is not properly set, gentle filing of the contacting edges of each half usually helps.

5) On completion of the setting, make sure that all the pins are exactly perpendicular to the anastomosis ring plane.

These pins might have been bent during the setting process. If so, these should be carefully straighted out with the special forceps.

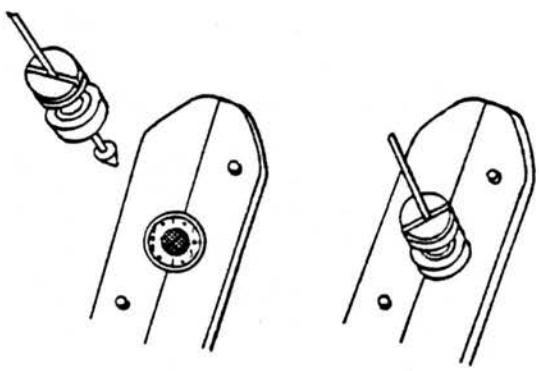
6) The intima of the vessels end is carefully everted and fixed onto the pins with the forcepses and fine hooks. Human vessels are often markedly sclerotic. The following technique is recommended in such circumstances. The vascular end is evenly pulled in outward direction with the three fine hooks evenly spaced on the intima at the vascular end, and the tissue is brought over the anastomosis ring to be hooked to three pins in an equilateral triangle fashion on every other pin. While two of the three points thus hooked are held down with the forcep, the intima between two points is hooked to the unased pin between them. This procedure is carried out on the two remaining unused pins (fig. 4.).



(fig. 4)

Complete eversion of the intima is thus attained with little injury to the intima.

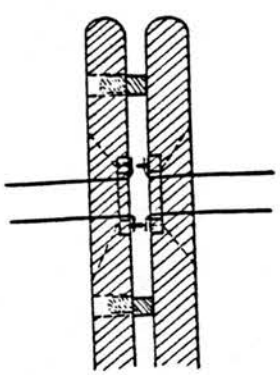
Then the thruster is applied on the vascular end that is everted on the vascular anastomosis ring in order to secure neat and deep fixation of the intima by the pins (fig. 5. a—b)



(fig. 5) a b

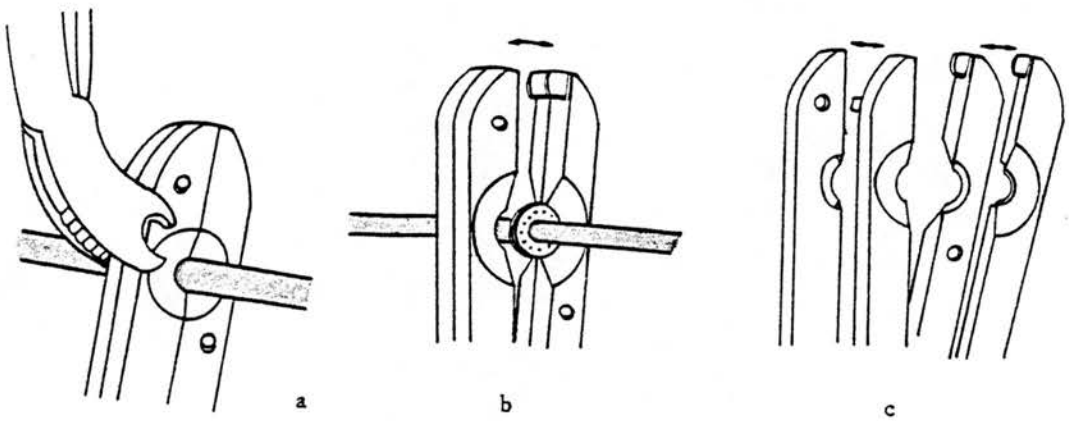
7) After both vascular ends are set on the two anastomosis ring, the anastomosis clamps are joined together by sliding the pins or process of one clamp into the corresponding holes on the other clamp (fig. 6).

8) The two clamps are then pressed on together with a pair of pliers. Pressing should be applied a few times around the ring-set portion to secure perfect cooptation.



(fig. 6)

9) As shown in figure 7, the two clamps should be opened while the upper side of both clamps is being held with the pliers. Then with the release of the clamps the anastomosis is completed (The rings become free from the vessel in a few weeks)

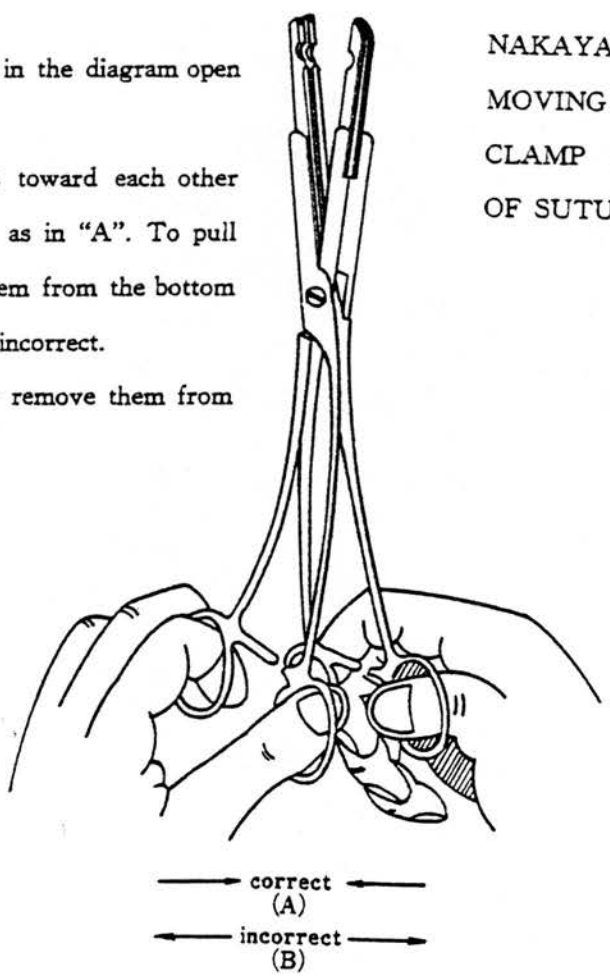


(fig. 7)

Holding the clamps as in the diagram open the scissors.

Then push the handles toward each other separating the top first as in "A". To pull the clamps separates them from the bottom first as in B. This is incorrect.

After separation simply remove them from the vessel.



NAKAYAMA'S METHOD OF REMOVING THE ANASTOMOSIS CLAMP AFTER COMPLETION OF SUTURE